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[INDEX]

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Emphasis in Cancer Research[1]

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The philosopher and physician, Albert Schweitzer, has characterized an outstanding attribute of modern Western civilization as the Will to Progress (*1*). The conviction that man can now improve his well-being on earth is widespread and so deeply ingrained that it is not questioned. This spirit deeply pervades research in cancer today. It is the basis for the wide public support in this area. The historical evidence of progress already made and a critical evaluation of current achievements give strong support, if not a guarantee, to the idea that this problem can be solved. The question is not whether, but in what way and how soon. This enemy will not yield to maneuvers in time and space; it must be attacked to be conquered. The comments which follow are intended to focus attention on the question of the optimum directness and emphasis of the attack.

The different varieties of cancer, collectively, are widely recognized as constituting a major health problem. The public has responded to the solution of this problem since the second World War by its interest and its support of research, contributing generously to private agencies and submitting ungrudgingly to taxation. The questions arise as to what direction the research effort is taking, and how near it is coming to the core of the problem. What is the target and how near are we coming?

In a previous study, an attempt was made to evaluate the size of the total cancer problem (*2*). It was estimated that about 300,000 new cases occur each year in the United States, that approximately 10 percent are cured, that an additional 20 percent could be cured by full application of all presently available knowledge on case detection, diagnosis, and treatment, and that the remainder, or 70 percent, represent the size of the research problem. The present analysis is a sequel to that paper inasmuch as it attempts to analyze the effort being expended in the solution of the remaining 70 percent.

Other evaluations of the research effort in cancer have been made by Deignan and Miller (*3*) who studied the amount of financial support, the

[1] Received for publication January 15, 1954.

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American Cancer Society which recently compiled a list of research projects (*4*), and others. Penetrating analyses of current trends have been made in cancer research by the Sixth Annual Report of the Committee on Growth (*5*), and in biological research by Weiss (*6*). The present study is not concerned with the amount of research in cancer or with its quality, but with its content and emphasis in relation to the essential problem. This implies first a judgment of what the problem is. One evaluation is presented, and it is followed by a classification of the research being performed in an attempt to study the emphasis of effort.

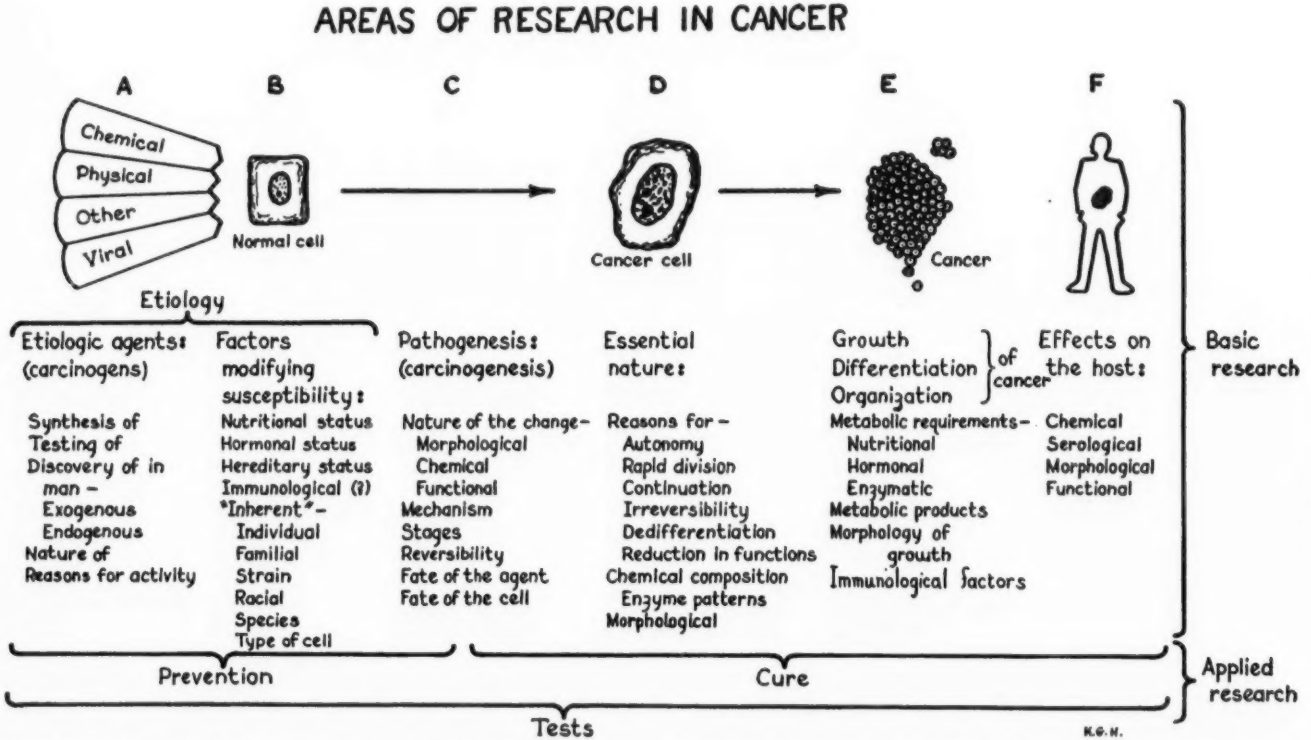
The Research Problem in Cancer

A concept of the research problem in cancer is given in text-figure 1. Cancer is induced by the action of certain carcinogenic factors (column *A*) on a normal cell (column *B*) as the consequence of which it is converted to a neoplastic cell (column *D*). By the proliferation of these new, altered cells, tumor masses are formed (column *E*), which exert their effects on the host (column *F*).

The factors that act on normal cells to transform them to cancer cells are called carcinogens. They are the etiological agents for the cancers they induce. The causes known today for human cancer (column *A*) include certain chemical and physical agents (*7*), plus a host of miscellaneous situations in which a cause-and-effect relationship is recognized but in which the active component is not yet identified. To these human carcinogens must be added, in the lower animals, certain viral etiological agents as well as many additional chemical and physical carcinogens (*8*).

Investigation in this area, which has been very active during the past two decades, has been concerned chiefly with the synthesis and testing of new chemical carcinogens in an effort to explain carcinogenic activity on the basis of molecular configuration and composition. Much less effort has been expended on the search for carcinogenic agents in animal tumors themselves, and still less work has been done on possible etiological agents in man and his tumors. The known carcinogenic agents together account for only a small proportion of all human tumors; the causes for the remainder remain to be discovered and they are presently called "spontaneous."

It has long been known on the basis of observation and experimentation in man and animals that all normal cells are not equally susceptible to conversion to a neoplastic cell by the action of inducing agents. Certain factors, many known and others unknown, govern the ease with which the reaction from column *B* to column *D* takes place under the influence of the agents of column *A*. The factors recognized as governing susceptibility, or resistance, to this neoplastic transformation include the hereditary composition and the ontological history of the cell, its nutritional state, its hormonal balance, and, possibly, certain immunological considerations. To these must be added other susceptibility-modifying factors which are less well understood; they include certain cellular, individual, familial, strain, racial, and species factors.



TEXT-FIGURE 1.—Areas of research in cancer. An outline of the research problem in cancer, used as the basis for classifying 1,000 research items and for the discussion on emphasis.

Whether or not a cancer will occur in a given case is a resultant of the two sets of etiological factors—the carcinogens and the susceptibility considerations. Cancer will probably occur if either or both are very potent, or if the resultant reaches a threshold level. If both are of a low order of potency or if one set of factors is absent, a tumor is unlikely to be initiated. Most of the known etiological agents are both exogenous and abnormal (*i.e.* pathological) in nature. The importance of endogenous carcinogens, of both physiological and pathological compounds, has also been a fertile field for investigation. The probability exists that certain normal compounds (*e.g.* hormones) acting under abnormal conditions may be carcinogenic. It is emphasized that the etiology of a disease is concerned both with the etiological agents and the host factors.

The pathogenesis of any disease is the way in which it evolves as the result of the action of the etiological factors. In cancer the pathogenesis is called carcinogenesis. Here are considered (column *C*) the mechanism of the change from normal to cancer cell, the morphological, chemical, and functional nature of the transformation, its stages, the question of reversibility during formation, the fate of the agent during this process, the immediate fate of the cell, the reactions of the host, and related aspects of the genesis of the altered new tumor cells. This area has been explored on a large scale in recent years since pure carcinogens became available.

Another large area in the research problem is concerned with the essential nature of the tumor cell (column *D*). Here interest centers on such things as the reasons for the high degree of autonomy of the cancer cell and its rapid divisions, the nature of the continuation forces for the abnormal cellular process, the reasons for its irreversibility, the failure of cell differentiation, and the reduction in cell functional output as contrasted with that of its non-neoplastic counterpart. Here also is found concern with the morphological and the chemical composition of the new cell and of its enzyme patterns as compared with the mother cell, and other problems.

After a cell has been changed from normal to neoplastic, its further multiplication to form a new protoplasmic mass, called a tumor, is under the control of a set of factors different from those that initiated the tumor. During this proliferation of the cells, which is abnormal (*i.e.* pathological), differentiation of the cells and their organization into tissues are also faulty. In this area of research (column *E*) fall such problems as the nutritional, hormonal, enzymatic, and other requirements for tumor organization, growth, and differentiation, as well as studies on the metabolic products, the morphology of the new protoplasmic mass, certain immunological considerations, the effects of the host on the tumor, and others.

The new cell mass, or tumor, is parasitic on its host, from which it derives its nutrition but to which it contributes nothing useful. As a result of its presence it may produce chemical, serological, morphological, functional and other effects on its bearer which have long comprised a large field of investigation (column *F*) in the clinic and laboratory.

It is well known from historical precedent in medicine that a disease

can be controlled in a population either by prevention, by cure, or by a combination. To these should be added the partial control provided by palliation of good quality. Most of the great victories came by prevention, particularly in the infectious diseases. Prevention can be accomplished by withholding exposure to etiological agents, by augmenting the host resistance factor, or by interfering with pathogenesis (columns *A*, *B*, and part of *C*). In human cancer, prevention is already practiced on a small scale, chiefly in occupational exposures, but not enough is yet known about the etiological agents, the cell susceptibility-modifying factors, the mechanism of the initial cell change, and other aspects of the pathogenesis to practice prevention on a large scale in the major types of cancer. Theoretically, carcinogenesis should be susceptible to stoppage up to some undetermined point in the arrow of column *C*, beyond which the outcome would be inevitable. There is some experimental substantiation for this idea. Prevention of disease may follow an understanding of either etiology or pathogenesis. Irradiation-induced sarcomas of bone can be prevented by withholding the etiological agent, smallpox by fortifying the host resistance factor, and myxedema by interfering with pathogenesis.

Once carcinogenesis has passed an irreversible point, a tumor is inevitable, and control of the disease must be accomplished by cure. At the present time this is done in the localized stages by removing the neoplasm surgically, or by destroying it *in situ* with ionizing radiations. For widely disseminated cancers only palliation can today be offered (with a few exceptions) and other means must be found for cure. Theoretically the possibilities include: *a*) methods for selectively seeking out and destroying the cancer cells but not the surrounding normal cells, as envisioned in radioisotope therapy and chemotherapy; *b*) methods for reversing the key abnormalities inherent in the cancer cell (column *D*) so that it is brought back into the society of normal cells and under the normal effective restraints; *c*) stopping the growth of the tumor cells by withholding components essential for nutritional and hormonal health; or *d*) increasing the host's defensive measures to the point where they equal or exceed the cell's aggressiveness.

The control of cancer in populations would be greatly aided if a simple and accurate test for susceptibility, for the incipient state, or for the early stages prior to dissemination were available as a screening procedure. The disease must be recognized before it can be cured. At the present time the chief reliance on definitive diagnosis is placed on morphological recognition (columns *D* and *E*) of the altered cells. In several types of cancer additional diagnostic tests are available which measure either some normal product of the tumor or some abnormal effect on the host. In the search for additional tests, all areas of research, from the identification of the etiological agents in column *A* to the effects of tumor on the host in column *F*, offer investigative possibilities. Identification of causative agents (as in tuberculosis) and of exceptionally high susceptibility (as in precursor groups), or recognition of the precancerous or the early cancer stages by chemical, morphological, serological, and other means all need to be

studied. The measurement of some property or product of the neoplasm or of some change in the host might be equally satisfactory as a test.

The subdivisions of research into basic and applied as indicated by the brackets on the right of text-figure 1 are approximate and not precise evaluations. Effort expended on prevention, cure, or tests may also be basic if it deals with fundamentals and principles as foundations upon which further investigation may take place.

In summary, the investigative problem in cancer can be conveniently divided into the conventional areas used in studying other diseases. These include etiology (both exogenous and host factors), pathogenesis, essential nature, and the effects on the host. The objective is either prevention or cure or both. Either would be greatly advanced by simple, reliable screening or diagnostic tests.

It is emphasized that, philosophically speaking, the research problem in bringing the disease cancer under control is not essentially different from that in other diseases such as typhoid fever, arteriosclerosis, or arthritis. They conform to the general outline in text-figure 1. The problem can be subdivided into the same broad categories. Cancer is not something apart and different, even though an understanding of its nature introduces the special disease concept called neoplasia. The basic principles of attack on the problem are not unique.

Present Emphasis in Research

The present research effort in cancer was analyzed according to the concept of areas just presented. The categories in text-figure 1 were supplemented by others whose nature and justification are readily apparent without definition.

To determine the present emphasis in research, it was thought sufficient to classify a large and representative sample rather than all existing work. For this purpose, four sources of information were used. First, all applications for research grants made to the National Cancer Institute and reviewed by the National Advisory Cancer Council in 1952 were classified. The undergraduate teaching grants were excluded but all other applications in the Research and Control Branches were used. Some of these proposals involved two or more areas. When they did, they were recorded in each of these categories; a few applications were thus classified in three or four different places. The total number of items so obtained was 617.

Next, all papers published during 1952 in three journals devoted to cancer were classified by the same method. The three were *Cancer*, the *Journal of the National Cancer Institute*, and *Cancer Research*. In the latter, the April number, giving only abstracts of the papers presented at the annual meeting of the American Association for Cancer Research, was eliminated because most of these investigations are reported *in extenso* in this journal or elsewhere. Together the items from these three sources numbered 383 as classified. The sum from all four sources totaled 1,000.

The applications for grants to the National Cancer Institute provided

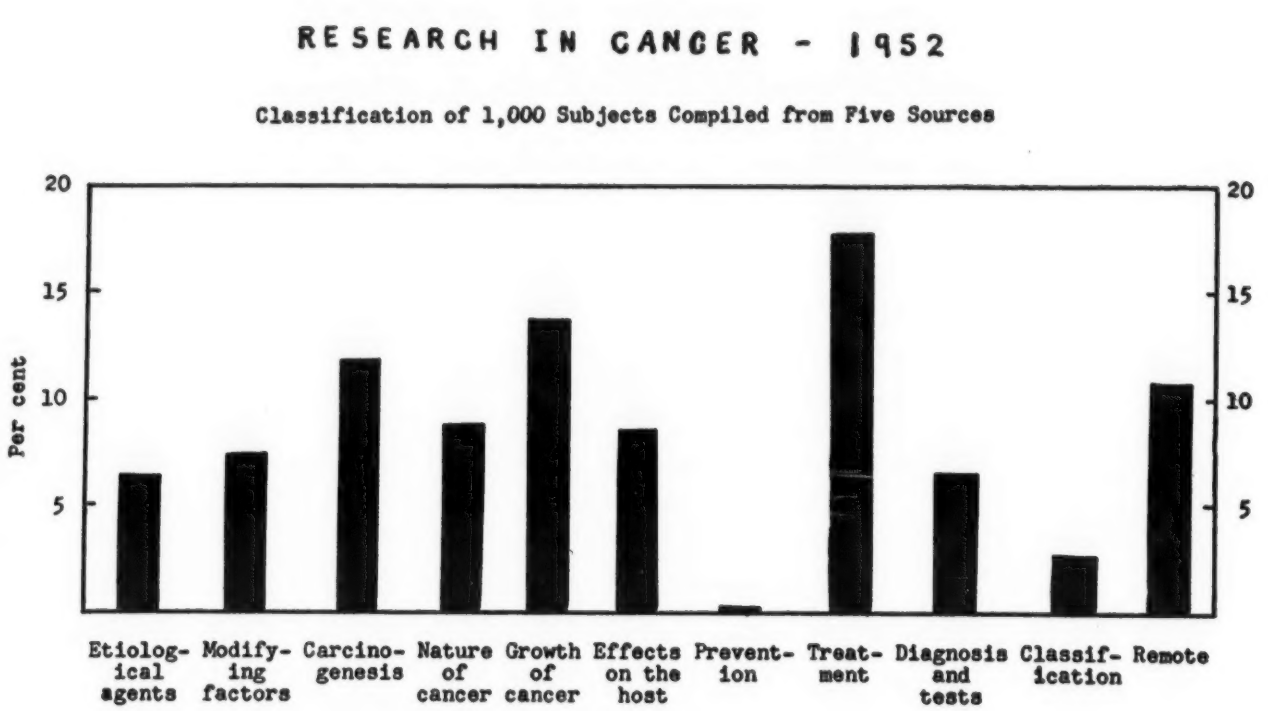
data on what ideas the investigators thought were important, what they wanted to do, or what they hoped would get a grant. It is emphasized that these figures represent applications and not approved grants, although it is believed that a classification of the latter would have come out relatively the same. The papers in the three journals represent research already accomplished which the investigators thought sufficiently important to warrant publishing.

The articles published in the three special cancer journals give a balanced cross section of research support in this field. They represent investigations supported by the following sources: the American Cancer Society through the Committee on Growth, its institutional grants, and the state and local affiliates of the Society; the intra- and extra-mural activities of the National Cancer Institute; the Jane Coffin Childs, Damon Runyon, and other funds; some university, governmental, industrial, and other institutionally supported research; privately financed work; investigations not supported by special grants; and other categories of effort. It is possible that the inclusion of investigations published in other journals in the basic sciences (genetics, physiology, botany, chemistry, zoology, etc.) would have altered the distribution and added to the category called remote. However, these papers could have been balanced by the inclusion of subjects from medical, surgical, pathological, and radiological journals, reporting on clinical investigations. It is believed that a fairly representative sample of the research effort in this country was sampled and classified. A change of a few percent in any direction would not greatly alter the evaluation and the deductions which follow.

The percentage distribution of the 1,000 research items classified from all sources is shown in text-figure 2, and in table 1. The largest area (17.9 percent) was treatment; it included much basic work in experimental chemotherapy, in toxicology, in pharmacology, and in related fields. Investigations on growth of cancer (13.8 percent), carcinogenesis (11.7 percent), nature of cancer (8.6 percent), and effects on the host (8.5 percent) followed in that order of frequency.

The considerable differences in percentage distribution of the items according to source of the data, revealed in table 1, reflect the special interests of these four sources. For example, the special functions of the Cancer Control Branch of the National Cancer Institute are shown by high percentages in investigation relative to diagnosis and tests, to educational efforts, and to treatment. Likewise, the high percentages for treatment, improvements in classification, effects on the host, and in diagnosis and tests reflect the interests of the journal *Cancer*. The journal *Cancer Research* shows a heavy interest in the growth, nature, and genesis of the disease, with emphasis on the chemical aspects.

The results show that the research attack is broad, covering all of the major areas. The general problems in cancer are clearly delineated although the exact methods to solve them are not always so clear. This may account for some of the inequalities in the proportions and balance of the work. For example, the subject of prevention, important as it is, has



TEXT-FIGURE 2.—Research in cancer—1952. A graphic percentage distribution of research items classified according to the outline given in text-figure 1 and in greater detail in table 1.

TABLE 1.—*Percentage distribution of 1,000 research projects in cancer—1952*

Source	Etiological agents	Modifying factors	Carcinogenesis	Nature of cancer	Growth of cancer	Effects on the host	Prevention	Treatment	Diagnosis and tests	Education	Surveys	Classification	Techniques	Remote	Miscellaneous
Cancer	0.8	2.3	7.0	6.2	12.5	10.9	0.0	24.2	10.9	0.8	1.6	18.8	.....	3.9	.....
Cancer Research	4.4	10.0	16.9	15.6	25.0	7.5	0.0	8.7	1.9	0.6	0.0	0.0	.....	9.4	.....
J. Nat. Cancer Inst	8.4	10.5	23.1	10.5	10.5	4.2	0.0	13.7	5.3	1.1	0.0	2.1	.....	10.5	.....
Research Branch, N. C. I.	6.1	8.5	9.4	8.3	14.2	10.6	0.0	21.1	3.7	0.6	0.0	0.4	1.6	14.8	0.8
Control Branch, N. C. I.*	14.7	1.8	10.1	0.9	0.0	0.9	1.8	12.8	22.9	22.0	7.3	0.0	1.8	0.9	1.8
Combined averages	6.3	7.4	11.7	8.6	13.8	8.5	0.2	17.9	6.6	3.0	1.0	2.8	0.9	10.6	0.6

*Teaching grants omitted.

as yet received only a small proportion of the total research effort. Expansion awaits additional information from other areas.

The balance between and within the categories becomes less impressive if the items are examined in detail. For example, studies on the etiological agents comprised 6.3 percent of the total. However, most of these studies were on animal tumors and on viruses as etiological agents. Only a small percentage were concerned with human etiological agents; and most of these were statistical and survey studies attempting to reveal clues to initiating causes. Thus, they are one step away from the agents themselves. Actually there was little work on possible etiological agents in man. Little effort is being made to determine to what extent, if any, the vast amount of information on experimental carcinogens applies to the human disease.

Comments on Emphasis

The research attack was found to be broad, covering all known major points of attack. Although we are not here concerned with depth, this also appears to be plumbed insofar as present perception goes. It would appear that the attack needs deepening rather than broadening at this time. The relative proportions and balance of the attack can be questioned but here one gets on highly controversial grounds. Insofar as the mature investigator is concerned, one is inclined to agree with Moe (*9*), with Kidd (*10*), with Richter (*11*), and others, that he must have freedom to follow his ideas with minimum interference, trusting that, once he has been designated as worthy and mature, he is best able to judge what is best.

On the other hand, the student, groping for understanding and probing for a sphere of promising interest in the field, is highly susceptible to outside influences. These can be either optimal or suboptimal, wise or unwise, and near- or far-sighted. These designations involve judgments on value, importance, and proportion. Good balance should be achieved, however, by education and not by coercion. It is with this understanding that the question of balance is raised, and it is in the light of the foregoing statements that the opinion is expressed that the present balance in cancer research is not optimal. This statement requires explanation.

Underemphasis on Etiology

An outstanding fact in table 1 and text-figure 2 is the small proportion of emphasis on etiology. Only 6.3 percent of research is being directed at the initiating causes of cancer. The total effort on etiology (6.3 plus 7.4 percent) is about 13.7 percent. Much more time, money, and thought are being devoted to the mechanism of formation (11.7 percent) and to the properties and growth of the tumor (8.6 plus 13.8 percent) than to etiology.

The reasons for this apparent imbalance are many. Some persons who do not distinguish between pathogenesis and etiology think they are working on the causes of cancer when actually they are working on its

pathogenesis—the way it evolves from the action of the causes. Others, who are concerned with the essential nature of the cancer process or of its growth, erroneously believe they are working on its causes. If the concept of the cancer problem as outlined in text-figure 1 and in the first section is valid, these distinctions are more than an exercise in semantics. Still other persons avoid the field of etiological agents for human cancer because of its limited beginnings in occupational cancer. They fail to realize that the problem of carcinogens has become one of environmental agents involving, in many instances, many if not all persons in some populations. This is an aspect of etiology that has hardly been touched, except peripherally by statistical methods. For example, the first known etiological agents for human lung cancer (tar fumes, asbestos, chromates, mixed ores, etc.) were confined to small occupational groups but the etiological principle that they established, namely, that human lung cancer can be caused by inhaled atmospheric agents, is now being applied to entire populations, with the emphasis on the carcinogens in soot and other anthracotic materials including tobacco products.

Differences in Research on the Normal and on Disease

Finally, there is unawareness among some cancer investigators of the etiological concept that has historically been so profitably used in the study of disease. The approach of the investigator to research is often determined by his past training and interests. Philosophically this is reflected in biology by an essential difference between the approach to studies of the normal (anatomical, physiological, and biochemical) and the abnormal or diseased (pathological) states. Thought on the normal tends to go immediately to mechanisms and essential nature: there are no undesired etiological agents. The scientist trained exclusively in the normal is usually not etiologically minded because he has not been concerned with etiology in the sense that it is used in pathological states. Study of a disease, on the other hand, begins with etiology. Practically every textbook description and lecture begins with a discussion of etiology. If cancer were a normal process, this particular problem regarding emphasis would not exist. Since cancer is not a normal process, the problem does exist as revealed by the present analysis. It would be desirable, since we are studying a disease, to emphasize if not actually to begin with first things, in this case etiology. The remedy may be to train the basic scientists to think etiologically if they elect to do research in disease.

The problem of determining what is the optimum research attack in disease is further complicated by two simple but fundamental differences between the normal and the abnormal biological worlds. The abnormal is quantitatively much larger than the normal, and it is qualitatively more complex. It is larger to the degree of the great number of pathological states that exist. There is within each person only one normal anatomical, functional, and biochemical range for each part, with allowances for the variations that occur with age and functional states. But matching each of these normals there exist an almost infinite number

and variety of pathological-anatomical, pathological-physiological, and pathological-chemical states. Thus for each type of normal liver cell, there is a great range of diseased liver cells, including many types of neoplastic cells, showing an equally great range in abnormal anatomy, chemistry, and physiology. Many of the variants in disease are already known because they have been studied either primarily or during investigations of the normal through the technique of using the abnormal for contrast, but most of them are incompletely understood.

The great range and variety of the abnormal in the various diseases and even within disease categories, such as the hundreds of kinds of neoplasms, creates an enormous research problem if the objective is to understand all aspects of every one of them. In contrast, the approach to a disease via its etiology may greatly simplify and reduce the size of the problem. The objective in cancer is conquest. Historically, control of diseases has been best accomplished by controlling etiology rather than by fully understanding the intricacies of nature and mechanisms. Even the recent great advances in control of disease by cure, as exemplified by the antibiotics, sulfonamides, vitamins, and hormones, did not come about by detailed understanding of mechanisms of the diseases concerned but often, as in pneumonia and other specific infectious diseases, by attacking the etiological agents involved.

Probably most kinds of cancer have more than one etiological agent (*e.g.* lung, bone). The situation for most tumors at the present time seems to resemble that in skin cancer, where one main etiological factor is probably responsible for most of the tumors and many agents for the remainder. The analogy with pneumonia is self-evident. The number of major causes in cancer appears, nevertheless, to be much fewer than the total number of cellular chemical abnormalities that could be studied one by one. Quantitatively and qualitatively, etiology in cancer appears to be simpler than mechanisms and the essential nature of the aberration.

Progress by Study of the Natural History of Diseases

Much progress in the conquest of diseases has come from the study of their natural history. Scurvy was controlled after it was observed to occur on ships in the absence of certain foods. Smallpox was controlled by vaccination of populations with cowpox after its protection of milkmaids was noticed. The observation that acromegaly was associated with eosinophil-cell adenomas of the pituitary indicated the existence of substances produced by ductless glands and led to the discovery of the hormones and the control of some diseases. During World War II filariasis became preventable in military personnel by using the knowledge on the short flight range of the microfilarial-infected mosquito, which followed an observation on the infection of people on one side of a small bay but not on the other. The morphological and clinical aspects of the natural history of the different cancers have been intensively studied during the last half-century and they are quite well understood. The etiological aspects of the natural history as revealed by statistical, geographical,

ethnic, hereditary, epidemiological, and other techniques are only recently coming under systematic study. The chief benefit to be derived from these studies is to reveal clues to etiology that can be taken to the laboratory for verification. The research problem originates from an observation on the natural disease.

This technique is hardly used at all today in cancer research. It may be seen applied in clinical medicine, pathology, and in epidemiology. Much of it is not grant-supported. It is represented in table 1 by only a few items. Its use requires a thorough understanding of the natural disease that the investigator is studying. The knowledge that a chief etiological factor in human skin cancer was solar irradiation came from studies on its site distribution, and its occupational, geographical, and racial frequencies, and this antedated confirmation in the experimental laboratory. It is indisputable that this method of investigating disease has been highly productive. Its small use in cancer research today is regrettable. There should exist a plethora of promising ideas in this area. Although the chief benefits may accrue to etiology, they could also lead to cure. The rationale for modern excisional therapy in esophageal cancer came from old studies showing the relative lateness of metastases in this variety of tumor in which obstruction of an essential passageway is early produced.

Essential Nature of Cancer

Cancer appears to be a reaction-type to many inducing factors, most or all of which are injurious short of lethality. Instead of reacting to these agents by mobilization of a special set of defense cells as in the infectious diseases, in cancer the reaction is made by the exposed cells themselves and in such a way that they are fundamentally altered. These changes are evident in their morphology, function, and chemistry. Jointly these features concern the pathogenesis and the essential nature of the disease and they constitute a large part of the research problem. Their understanding might lead to checking or reversing the abnormality.

The cancer process had been or is now being characterized at morphological, functional, and biochemical levels. A satisfactory characterization of its essential nature cannot be made in terms of any one or two of these alone. A composite of all three is required to give a satisfactory comprehension of the neoplastic process. The research worker in cancer should be specialized enough to develop and exploit new areas but broad enough to incorporate his findings into the total concept.

Cancer More Than a Problem in Growth

Although no data were compiled in this study on trends in emphasis, it is apparent to those who have followed the papers in the three cancer journals already mentioned over a period of years that changes have occurred. Conspicuous among these is the increasingly greater emphasis on the growth of cancer. The figure of 13.8 percent of all items reviewed assigned to this topic reflects recent expansion. There appears to have

been no corresponding increase in the work on differentiation and organization in cancer although these basic cellular attributes are equally as faulty as growth. Historically, this trend may be attributed to the recent emphasis, if not overemphasis, on characterization of the cancer problem as primarily one of deranged growth.

Used with the broadest figurative connotation, the abnormal growth in cancer is only one among six or eight problems of equal size if not of importance (text-fig. 1). Used in the literal sense, the statement becomes frankly misleading. The attractive statement that the problem of cancer is primarily a problem in growth is both partly true and partly untrue, but it is incomplete and oversimplified. It has become a cliché that needs critical analysis.

The point was previously made that organization and differentiation are fully as defective as growth: Furthermore, the ultimate sought-for conquest of cancer by prevention may have nothing to do with the growth of cancer cells, while the control by cure has equally little concern with the essential nature of abnormal growth in the localized forms. In the generalized forms, concern with growth will exist only if a biological cure becomes imperative for want of some other method. Even then an understanding of all the basic phenomena in metabolism and growth would not necessarily be essential. The great advances gained by chemotherapy in the infectious diseases through the discoveries of Ehrlich, Domagh, Fleming, Waksman, and others do not rest on a complete understanding of the growth of the microorganisms that they control or the pathological tissues that they induce (*e.g.* the gumma), or on how the drugs interfere with growth. Neither does the basis for the present achievement—curability of about 30 percent of all cancers (*2*)—rest primarily on research in growth even as the term is used today. If the outline as here proposed is valid (text-fig. 1), the cancer problem is not properly designated as one primarily of growth, and the true growth aspect is only one among many other facets. The young investigator, searching for a promising field of interest, ought to understand this very clearly.

The emphasis on the growth aspects of the cancer problem has resulted in much valuable progress which is not reviewed here. One interesting advance might be mentioned. This is the ability to maintain some cancers alive in heterologous hosts or in tissue culture almost indefinitely and long after the original bearer is dead. Man is still mortal to cancer but his tumor is immortal! Man is interred but his tumor remains alive—surely a curious reversal of the desired goal and, one hopes, a temporary way-station toward the ultimate objective.

The Question of Remote Research

The category of subjects designated as "remote" (10.6 percent) deserves special comment. It contains some of the finest work. Items of two distinct types were included in this category. Both failed to qualify under any of the other headings because of no apparent direct relation to

cancer, but from this point they diverged. In one group, the remoteness was a part of a plan leading ultimately back to cancer. The remoteness was an investigative device, elective or necessary, to gain information or a base line by studying normal, embryonic, hypertrophic, hyperplastic, atrophic, hypoplastic, or other pathological states. In the other group, the investigator was interested in basic problems, usually biological or chemical, which had no immediate or presently apparent bearing on cancer and which were not a step in a cancer investigation.

It is emphasized that the characterization as remote had nothing to do with the basic nature of the research. It was not a question of basic versus applied investigations. There can be no question about the need for basic research. Rather, the problem was one of basic research in cancer versus basic research in other fields.

It appears to be historically correct to say that once a research has been designated as remote, its possible contribution to cancer, or to any other specific problem in disease, cannot any longer be judged. Many of the greatest advances in cancer control have come from research that had no thought of benefits in cancer. The physicist, Wilhelm Roentgen, was not concerned with cancer during his experiments with the rays that bear his name, and Landsteiner laid the foundation for modern blood transfusions, so necessary for cancer surgery, in his work on blood groups. Other examples could be cited to show that much of the best work has come from the remote category. Some remote research has suddenly become proximate.

These facts present a practical problem to administrators of cancer research funds which has not been solved. These categorical funds are not large enough to support all desirable basic research in the biological and physical sciences. This important problem deserves far more consideration than can be given here. The view was previously expressed that if it comes to a decision between two excellent proposals both equally fundamental in nature but one apparently remote and the other proximate to cancer, that the administrator of cancer funds is almost bound to decide in favor of the latter (*2*). This conclusion is not entirely satisfactory, and further exploration of the problem is in order. One appears to be on safe grounds in stating that basically good research on a cancer problem ably performed by a sound investigator is certain to further cancer work, while an equally good investigation in the remote category may or may not contribute to the field of cancer. On the other hand it may prove of value in some other specific problem, but this is true also of the research in cancer. The important unanswered question is whether support in the remote category will add up to more progress in the long run than an equivalent amount of effort in the proximate.

In touching upon some of these important problems, there is danger of being misunderstood or accused of bias. This risk must be faced if these problems are to be aired. There is wide agreement on the necessity for biological research on the broadest and deepest possible scale. The points of view here expressed are fully in agreement with that view. The addi-

tional point made here is that if the objective is conquest of a specific disease, a direct attack is equally possible and it may even be more desirable. The point is further made that cancer appears to be such a disease. In science, progress is made by driving out special salients which must then be related to the whole body of knowledge to roll forward a front. A plexus of disorganized salients if not exploited benefits no one and can lead to confusion. It is only proper to point out that in accepting resources allocated to a specific disease problem, the investigator assumes a responsibility to relate his special salient to the advance of the whole.

It is well known that much basic understanding, some good palliation, and additional promising information toward cures have come from research aimed directly at cancer problems. It is not necessary to aim at remoteness to achieve progress.

Résumé

An outline of the research problem in cancer is given in text and figures. If this conception is essentially correct, the problem can be outlined relatively simply, although the exact technical methods to achieve the goals are not. The research problem can be simply expressed according to the same categories that have historically been so fruitful in other diseases. These general areas include the two components of etiology as distinguished from the pathogenesis and the essential nature of the disease; and its diagnosis, treatment, etc. An analysis of the present research effort, based on a study of 1,000 items in 1952, reveals it to be broad, covering all of the principal areas. The balance in the emphasis can be challenged as less than optimal, but this is a subjective appraisal. The opinion is expressed that studies on etiological factors, which might lead to cancer control by prevention, are being underemphasized in relation to those on the already established tumor. Some basic differences are pointed out in the research approach, as it is now practiced, to studies of the normal as contrasted with pathological states. Up to now, the conquest of diseases has been achieved more easily by gaining control of their etiology than by a full understanding of their essential nature. An understanding of the natural history of the disease under investigation has often contributed important ideas toward its control. The use of this method is now at a low ebb in cancer work. The view is also expressed that cancer is much more than a problem in growth, and that the recent frequent characterization of the cancer problem as one of growth is an oversimplification in the figurative sense, and misleading in the literal sense. The problem of emphasis in areas designated as remote and proximate in the attack on specific diseases is re-considered. This does not involve a question of basic versus applied research. As in other areas in science, research effort tends to go into the most promising fields, and no element of coercion is desirable to secure readjustment. Any desirable change in emphasis in cancer should come by the balanced education of students and investigators in the essential problems.

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Effect of Growth of Mouse Sarcoma 37 on Resistance of Mice to Semilethal Doses of Certain Trivalent Arsenic Compounds[1]

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In the course of determining lethal dose values for certain arsenic compounds (*1*), it was noted, for one of the compounds, that the mortality rate was much less for mice bearing Sarcoma 37 than it was for normal (non-tumor-bearing) mice. This led to an investigation of comparative mortality exhibited by normal and tumor-bearing mice following administration of various trivalent arsenic compounds.

Materials and Methods

Solutions of arsenic compounds injected subcutaneously into mice.—Numbers employed to designate compounds or preparations are those of the order of accession of these compounds or preparations by the Laboratory of Chemical Pharmacology, National Cancer Institute. 3-Arsenonitrobenzene (#822) and 3-arsenosoanisole (#824) were administered dissolved in olive oil. Solutions of arsenite (#2174) of *p*H about 9.6 were secured by diluting stock Fowler's solution, prepared as described in the U. S. Pharmacopoeia. Solutions of all other arsenic preparations employed were secured by dissolving them in water or dilute NaOH. Except when stated otherwise, the solutions were at or near *p*H 7. The arsenic compounds and preparations employed in aqueous or dilute alkali solution were as follows: #118, 3-acetamino-4-hydroxyphenylarsenoxide; #190, 3-amino-4-hydroxyphenyldichlorarsine HCl, solution of *p*H 1.7; #190C, consisting of one part by weight of compound #190 plus 3.3 parts by weight of a Squibb proprietary citrate-acetate buffer, solution of *p*H 5.5 (designated as Clorarsen by Squibb & Sons); #192, 3-amino-4-hydroxyphenylarsenoxide (Parke-Davis mapharsen), solution of *p*H 3; #192C, consisting of one part by weight of compound #192 plus 4.3 parts by weight of the Squibb proprietary citrate-acetate buffer, solution of *p*H 6.5; #828,

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5,5′-diamino-2,2′-dimethoxyarsenobenzene-N,N′-dimethylarsenobenzene-N,N′-dimethylene disodium sulfonate; and #829, 5,5′-diamino-2,2′-dimethylarsenobenzene-N,N′-dimethylene disodium sulfonate.

The compounds were employed at concentrations such that the desired doses, in mg. per kg., were secured by subcutaneous injections into mice of 0.01 ml. of solution per gram of body weight.

Plan of experiments to compare resistance of normal and tumor-bearing mice to lethal effects of arsenic preparations tested.—In those experiments in which comparison of the death rate in a group of normal (non-tumor-bearing) mice with the death rate in a group of tumor-bearing mice was to be made by the Chi-square test (*2*), the dose of the test arsenic compound was a dose that previous experiments (*1*) had indicated would kill more than 50 percent and less than 100 percent of normal mice. In these tests, two groups of 20 or more mice were used, one group of normal mice and one group of tumor-bearing mice. If comparison was to be made on the basis of LD_{50} values, as determined by a variation of Karber's method described by Cornfield and Mantel (*3*), doses were employed that covered the entire range from 0 to 100 percent mortality. The determinations of the comparative LD_{50} values involved the use of 50 or more normal mice and 50 or more tumor-bearing mice (table 2, expts. 2 and 3).

In both Chi-square and LD_{50}-type experiments, the tumor-bearing and non-tumor-bearing mice used in any one experiment were all of the same strain (or hybrid) and sex, and approximately the same age (2 to 4 months). The numbers of mice used in particular experiments are designated in the tables.

In the early experiments, approximate weight equality of tumor-bearing and non-tumor-bearing mice at the time of tumor implantation was secured by weighing each mouse prior to its assignment to the tumor-bearing or the non-tumor-bearing group. Later it was found that with the large numbers of mice being used, approximate weight equality was secured simply by selecting at random, for tumor implantation, half of the mice in each cage of mice employed in a given experiment or set of experiments. From the data of tables 1 and 2, it is apparent that in every experiment performed the body weights found for mice at the time of administration of an arsenic compound, 3 to 7 days after tumor implantation, were substantially the same for the tumor-bearing mice as for the non-tumor-bearing mice.

Mice and tumors used.—Increased resistance to trivalent arsenic compounds with growth of mouse Sarcoma 37 was most strikingly evident in experiments in which CAF_1 female mice were used [F_1s from BALB/c females $\times$ A males (*4*)]. For the sake of brevity, data of both positive and negative experiments with CAF_1 male mice, and with CFW (*4*) and ABC mice (obtained from Dr. J. J. Bittner, University of Minnesota) have been omitted. Tumors employed were Sarcoma 37, and Lymphoma #1 (*5*), transplanted by injecting tumor mash in saline intramuscularly in the right hind leg.

Chemical analyses for arsenic content of tissues in mice bearing Sarcoma 37, and injected subcutaneously with arsenic preparation #190C.—Female CAF_1 mice, bearing Sarcoma 37 implanted in the right hind leg 6 days earlier, were injected subcutaneously with a solution of arsenic preparation #190C in the amount of 47.3 mg. of compound #190 per kg. This corresponds to 12 mg. of arsenic per kg. Two hours later, 5 mice were sacrificed. Samples of tumor tissue, liver, kidney, and skeletal muscle were excised, pooled, weighed, and dried to constant weight at 100° C. Pooled dried samples of these same tissues were also obtained in the same manner from mice sacrificed at 7, 24, and 48 hours after injection of the arsenic preparation. Analyses for arsenic content of the dried tissue samples were made by the method of Magnuson and Watson (*6*).

Results

In all but one of twelve experiments in which arsenic preparation No. 118, 190C, 192C, 828, or 2174 was administered, the 48-hour mortalities found for CAF_1 female mice bearing Sarcoma 37 were significantly less than those for corresponding non-tumor-bearing CAF_1 female mice (tables 1 and 2). In ten of the twelve experiments, a difference in the same direction was found at 7 days after injection of the arsenic compound, and it was statistically significant in seven of the experiments. It would appear, therefore, that a genuine difference in resistance to semilethal doses of certain arsenic compounds exists between *a*) non-tumor-bearing CAF_1 female mice, and *b*) CAF_1 female mice bearing Sarcoma 37 implanted 3 to 7 days prior to administration of the arsenic compound.

More delayed deaths (deaths occurring between 2 and 7 days after administration of an arsenic compound) were noted for tumor-bearing than for non-tumor-bearing mice. However, an interesting relation is revealed on analysis of the pooled data in experiments 4 to 10 of table 2. In these experiments, in which a total of 181 non-tumor-bearing mice and a total of 181 tumor-bearing mice were employed, either 3-amino-4-hydroxyphenylarsenoxide or its precursor, 3-amino-4-hydroxyphenyldichlorarsine, was administered in a solution at *p*H 5.5 or 6.5. About 10 percent (6) of the 58 non-tumor-bearing mice still alive at 48 hours after injection of arsenic compound died in the 2- to 7-day period. About 15 percent (22) of the 143 tumor-bearing mice still alive at 48 hours after injection of arsenic compound died in the 2- to 7-day period. This suggests that the presence of the tumor protected mice against deaths occurring as a result of acute poisoning by the arsenic compound, but did not appreciably affect the likelihood of a delayed death occurring as a result of subacute poisoning. A complicating factor is the fact that Sarcoma 37 is a tumor that grows with unusual rapidity. By 13 days after tumor implantation (7 days after injection of an arsenic compound into mice bearing tumors implanted 6 days earlier) the tumors were typically larger than the head of a mouse, with raw and scabbed areas protruding through the skin, and the hosts appeared to be in poor health, *i.e.*, they exhibited lethargy, hyperpnea, and dirty and matted hair. With this

TABLE 1.—*Comparative resistance of* a) *non-tumor-bearing mice, and* b) *mice bearing Sarcoma 37, to semilethal doses of various arsenic compounds*

Expt. No.	Compound used		Mice used (CAF_1 females)			Percent of mice in group dead up to:		*P* values for group NT *vs.* group T§	
	No.	Dose (*mg./kg.*)	Group*	Number in group	Body weight range in group (*gm.*)	48 hrs. after As injection	7 days after As injection	At 48 hours	At 7 days
1	118	50	NT	25	20–29	52	76	0.01	NS
			T-6d.	25	19–29	8	56		
2	822	25	NT	30	19–26	73	73	NS	NS
			T-7d.	26	19–26	44	50		
3	824	50	NT	25	19–26	100	100	NS	NS
			T–7d.	25	19–26	80	88		
4	828	40	NT	25	21	64	.....	.03	.....
			T-6d.	25	21	24	.....		
5	828	40	NT	25	16–27	56	56	.04	.04
			T–6d.	25	16–25	24	24		
6	828	40	NT	40	16–26	55	55	.03	NS
			T-6d.	28	17–23	21	57		
7†	829	50	NT	24	14–21	79	79	NS†	NS
			T-6d.	26	15–24	50	81		
8	2174	10	NT	40	.....	60	63	$<.001$	$<.001$
			T-6d.	40	.....	10	13		
9‡	2174	10	NT	35	.....	31	31 (4d.)	.04 for NT *vs.* T	
			NT-P	18	.....	39	39 (4d.)	.02 for NT-P *vs.* T	
			T-6d.	20	.....	5	5 (7d.)		

*NT: Group of non-tumor-bearing mice, controls for tumor-bearing mice. T: group of tumor-bearing mice. The time between tumor implantation and administration of arsenic compound is indicated in days, *e.g.*, 6d.=6 days.

†In this experiment, even though no significant difference was present at 48 hours or at 7 days after injection of the arsenic compound, NT mice began dying much earlier than T mice—at 24 hours, 75% of NT and only 31% of T mice were dead.

‡In this experiment an additional control group consisting of 18 pregnant females, and designated NT-P, was used.

§NS: Not significant.

point in mind, it would appear legitimate to raise the question whether the considerable number of delayed deaths that occurred in experiment 6 of table 1, performed with compound 828, were due primarily to delayed arsenic poisoning or to deleterious effects of the rapidly growing tumors. If the former, it is difficult to understand why considerable numbers of delayed deaths did not occur in experiment 5 of table 1, performed with this same compound and at the same dose.

In experiment 9 of table 1 three groups of female mice were employed; namely, a group of mice bearing Sarcoma 37, a group of non-tumor-bearing nonpregnant females, and a group of non-tumor-bearing pregnant females. From the table, it is apparent that pregnancy did not result in a significant increase in the resistance of CAF_1 female mice to arsenite, whereas the presence of the growing tumor did induce an increase in resistance to arsenite poisoning.

The presence of Sarcoma 37 did not perceptibly affect the resistance of the mice to 3-arsenonitrobenzene (#822) or to 3-arsenosoanisole (#824), the two arsenic compounds that were administered dissolved in olive oil.

Influence of pH of the injected solution on the lethal effects of 3-amino-4-hydroxyphenylarsenoxide.—When 3-amino-4-hydroxyphenyldichlorarsine (preparations 190 and 190C) is dissolved in water, it hydrolyzes immediately to 3-amino-4-hydroxyphenylarsenoxide plus 2 HCl. Similar biological effects were therefore expected for arsenic preparations 190, 190C, 192, and 192C. However, LD_{50} determinations indicated that #190C was definitely more toxic to non-tumor-bearing female mice than to female mice bearing Sarcoma 37 (expt. 3, table 2), whereas preparation #192 was about as toxic for tumor-bearing female mice as for non-tumor-bearing mice (expt. 2, table 2). Preparation 190C was found to give solutions of *p*H 5.5, whereas preparation 192 gave solutions having *p*H values of 3.0. This indicated that the *p*H of the injected solution was an important factor in determining whether 3-amino-4-hydroxyphenylarsenoxide would exert a differential toxicity on tumor-bearing and non-tumor-bearing mice. Further experiments were therefore performed by Chi-square procedures (table 2, expts. 1, 4, 5, 6, 7, 8, 9). Data for all of the experiments, both those of Chi-square type and those of LD_{50} type, have been arranged in table 2 according to the *p*H of the injected solutions. It would appear that 3-amino-4-hydroxyphenylarsenoxide, administered subcutaneously in solutions at *p*H 1.7 or 3.0, was equally toxic to tumor-bearing and non-tumor-bearing mice. When administered in solutions at *p*H 5.5 or 6.5, this compound was significantly more toxic to non-tumor-bearing mice than to mice bearing Sarcoma 37.

Effects of age of tumor on comparative mortalities induced by 3-amino-4-hydroxyphenylarsenoxide in tumor-bearing and non-tumor-bearing mice.—Greater resistance against arsenic preparations 190C and 192C was exhibited by tumor-bearing than by non-tumor-bearing mice, in experiments in which the time between tumor implantation and injection of the arsenic preparation was 3 days (table 2, expt. 8), 4 days (table 2, expts. 5 and 9), and 6 days (other experiments, table 2). On the other hand, in

TABLE 2.—*Effect of pH of injected solution on comparative resistances of a) non-tumor-bearing mice, and b) mice bearing Sarcoma 37, to 3-amino-4-hydroxyphenylarsenoxide*

Expt. No.	Arsenic preparation used			Mice used (CAF_1 females)			Percent dead up to:		$LD_{50} \pm$ S. E. (*mg./kg.*)*		*P* values for group NT *vs.* group T§	
	No.	Approx. *p*H of solution	Dose (*mg./kg.*)*	Group†	Number in group	Body weight range in group (*gm.*)	48 hours	7 or 8 days	At 48 hours	At 7–8 days	At 48 hours	At 7–8 days
1	190	1.7	45	NT	20	.....	75	.....	.....	.....	NS	.....
				T-6d.	20	.....	80	.....	.....	.....		
2	192	3.0	25–80	NT	50	19–26	.....	.....	42.7 ±2.8	.....	NS	.....
				T-6d.	60	18–26	.....	.....	37.2 ±2.4	.....		
3‡	190C	5.5	20–63	NT	60	19–29	.....	.....	34.5 ±1.6	34.5 ±1.6	<.001	<.001
				T-6d.	50	19–29	.....	.....	51.0 ±2.7	49.2 ±3.5		
4	190C	5.5	46	NT	25	19–27	56	72	.....	.....	.001	.01
				T-6d.	25	21–28	0	20	.....	.....		
5	190C	5.5	46	NT	25	18–22	64	64	.....	.....	.03	NS
				T-4d.	25	18–22	24	44	.....	.....		
6	190C	5.5	46	NT	30	20–24	87	87	.....	.....	.03	.03
				T-6d.	31	19–24	42	42	.....	.....		
7	190C	5.5	46	NT	25	.....	40	48	.....	.....	NS	NS
				T-6d.	25	.....	24	44	.....	.....		

8	192C	6.5	40	NT	25	18–22	88	88	.....	.....	.005	.03
				T-3d.	25	18–22	28	40	.....	.....		
9	192C	6.5	40	NT	25	18–22	80	80	.....	.....	<.001	<.001
				T-4d.	25	18–22	8	16	.....	.....		
10	192C	6.5	40	NT	26	.....	54	54	.....	.....	.02	NS
				T-6d.	25	.....	16	24	.....	.....		

*Nonarsenic admixed compounds disregarded.

†NT: non-tumor-bearing mice; T: tumor-bearing mice. The time (days) between tumor implantation and injection of the arsenic preparation were as follows: Expt. 8: 3 days; expts. 5 and 9: 4 days; all other expts.: 6 days.

‡For expt. 3, LD_5 and LD_{50} values for NT mice were 24.7 and 48.4 mg. per kg., respectively; LD_5 and LD_{50} values for T mice were 36.5 and 71.2 mg. per kg., respectively.

§NS: not significant.

experiments not shown in the tables, using mice bearing Sarcoma 37 implanted 9 or more days prior to the injection of an arsenic preparation, tumor-bearing mice were usually *less* resistant than non-tumor-bearing mice to semilethal doses of arsenic compounds. This is perhaps not surprising, since by 9 or more days after its implantation Sarcoma 37 was often about the size of the head of a mouse, and the tumor-bearing mice appeared to be in relatively poor condition. Mice showed little palpable tumor tissue at 4, and especially at 3 days after implantation of Sarcoma 37.

Arsenic content of normal and tumor tissues, following administration of arsenic preparation #190C.—If trivalent arsenic compounds were preferentially accumulated in tumor tissue, this might bring about a decrease in concentration of the arsenic compounds in other tissues, just sufficient to induce a measurable difference in resistance of tumor-bearing and non-tumor-bearing mice to the compounds. The arsenic content of certain tissues of CAF_1 female mice bearing Sarcoma 37, found at varying time intervals after injection of arsenic preparation #190C, are shown in table 3. It is apparent from this table that this arsenic preparation, and/or its arsenic-containing derivative(s), is accumulated in liver and kidney far more than in tumor tissue. It would appear to be highly unlikely that the amounts of arsenic compound present in tumor tissue are sufficiently great at any time after injection of arsenic compound to bring about an appreciable decrease in concentration of arsenic compounds in other parts of the mouse.

TABLE 3.—*Arsenic content of mouse tissues at varying times after subcutaneous injection of arsenic preparation 190C (12 mg. of arsenic per kg.)*

Tissue	Arsenic content (mg. per kg. of wet tissue)					Average percent water in tissue
	Controls (no As injected)	Time in hours after injection of arsenic				
		2	7	24	48	
Sarcoma 37	0	2.9	2.6	2.1	0.3	82.3
Liver	0	13.0	11.6	1.8	0.3	71.9
Kidney	0	23.7	12.4	3.3	0.7	73.6
Skeletal muscle	0	2.8	4.0	1.6	0.0	71.6

Experiments with Lymphoma #1 (5).—Two experiments were performed in each of which the 48-hour percent mortality induced by arsenic preparation 192C (45 mg. of compound 192 per kg.) in a group of 25 non-tumor-bearing CAF_1 female mice was compared with the mortality induced by an identical amount of this same arsenic preparation in a group of 25 CAF_1 female mice bearing Lymphoma #1 implanted in the right hind leg. No appreciable or significant difference in mortality between tumor-bearing and non-tumor-bearing mice was noted. In one of the two experiments the time between tumor implantation and injection of arsenic

preparation 192C was 7 days, in the other 11 days. At 11 days after implantation, L #1 tumors were about the size of Sarcoma 37 tumors at 5 days after implantation. At 7 days after implantation, L #1 tumors were barely palpable.

Discussion

In work with a tumor-necrotizing polysaccharide derived from *Serratia marcescens*, Shear (*7*) noted that smaller doses were required to kill mice bearing carcinogenically induced sarcomas than to kill non-tumor-bearing mice. In numerous tests made over a period of many years in the Laboratory of Chemical Pharmacology, National Cancer Institute, it was observed repeatedly that a given dose of the polysaccharide induced a higher percentage of deaths in mice implanted 6 days earlier with Sarcoma 37, than in non-tumor-bearing control mice. Toxicity tests of a semiquantitative nature performed in the Laboratory of Chemical Pharmacology on many different organic compounds of widely varying chemical structure, and using mice implanted with Sarcoma 37 about 6 days earlier, generally revealed a greater toxicity in tumor-bearing mice than in non-tumor-bearing mice.[6] It would appear likely, therefore, that the greater resistance against semilethal doses of certain trivalent arsenic compounds exhibited by mice bearing Sarcoma 37, as compared with non-tumor-bearing control mice, constitutes a special case.

The findings of Dalton (*8*) that tumor growth is associated with characteristic histologic changes in the adrenal cortex has led one of us to investigate effects of adrenal hormones on the resistance of mice to semilethal doses of trivalent arsenic compounds (*9*). Upjohn Company beef adrenal extract and cortisone were found to have a protective action. However, in the experiments with adrenal hormone preparations greater protection was afforded male than female mice, whereas the presence of Sarcoma 37 afforded female mice greater protection than it did male mice. At present, we can only speculate that perhaps both the hormone preparations and the presence of Sarcoma 37 induced an increase in concentration in mouse tissues of sulfhydryl groups capable of detoxifying or speeding up the excretion of arsenic. It is interesting to note that Schacter, Entenman, and Shimkin (*10*) have observed a decrease in plasma sulfhydryl concentration during growth of the Murphy rat lymphosarcoma. This they interpreted to indicate an increased demand for sulfhydryl by the tissues during tumor growth. In view of the interdependent metabolism of ascorbic acid and glutathione (*11*), it is also interesting to note that Leise, Harvey, and Schwanfelder (*12*) have reported a significant increase in concentration of ascorbic acid in liver, kidneys, and adrenal glands during growth of certain mouse tumors.

Summary

1) Using CAF_1 female mice and Sarcoma 37, tumor-bearing mice were found to be more resistant than non-tumor-bearing mice to semilethal

[6] Personal communication from Dr. J. Leiter.

doses of certain trivalent arsenic compounds. Mice with very large tumors failed to exhibit greater resistance than non-tumor-bearing mice.

2) Greater resistance of tumor-bearing than of non-tumor-bearing mice to 3-amino-4-hydroxyphenylarsenoxide was exhibited when this compound was injected into mice in a solution brought to *p*H 5.5 or 6.5 with citrate-acetate buffer salts, but not when it was injected into the mice at *p*H 1.7 or 3.0.

3) The arsenic of 3-amino-4-hydroxyphenyldichlorarsine was accumulated in liver and kidney far more than in Sarcoma 37 or skeletal muscle.

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Nitrogen, Sulfhydryl, and Disulfide Contents in Normal and Cancer Sera and in Their Albumin and Globulin Fractions. Action of Sulfite on Disulfide Groups in Normal and Cancer Sera [1,2]

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In a recent publication (*1*) we reported, in agreement with results of other workers (*2*, *3*), that the sulfhydryl content of whole blood serum of healthy persons was considerably greater [average of 28 sera, 6.2 mg. cysteine per 100 ml. serum (*1*)] than that of sera from cancer patients [average of 67 cases, 3.8 mg. cysteine per 100 ml. serum (*1*)]. Our technique for the amperometric titration of sulfhydryl groups has been extended to the albumin and globulin fractions of blood sera. The results should make it possible to decide whether the difference in sulfhydryl content between normal and pathologic sera is partly or entirely due to a varying ratio of albumin and globulin and/or varying total protein content, or whether the sulfhydryl content of each of the two protein fractions is different in normal sera as compared with sera from cancer patients. While our work was under way, Schoenbach *et al.* (*4*) published an excellent paper on the sulfhydryl content of sera and of albumin and globulin fractions of sera from healthy and sick individuals. We could confirm most of the results reported by these investigators.

Since little information is available on the disulfide content of proteins in sera from normal persons or those with cancer, we determined the disulfide in these sera and also in their protein fractions by applying a simple procedure recently developed in this laboratory (*5*). In the course of these investigations, interesting observations were made on the reactivity of disulfide groups with sulfite in normal and pathologic sera.

Materials

All chemicals used were commercial C. P. products.

The mercuric chloride solution used for the determination of sulfhydryl groups was 5×10^{-4} *M*, and the silver nitrate solution used for the determination of the disulfide content was 10^{-3} *M*. Hypobromite solutions

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for the amperometric titration of ammonia were prepared according to directions described previously (*6*).

Experimental

Nitrogen, Sulfhydryl, and Disulfide Determinations

Details for preparation and storage of blood sera have been reported in a previous paper (*1*). The normal sera were obtained from healthy young workers in the School of Chemistry of the University of Minnesota. The pathologic sera were obtained from the Hospital of the University of Minnesota, taken from patients with definite diagnoses of cancer (confirmed by biopsy or operation).

The sera were fractionated according to the method of Pillemer and Hutchinson (*7*) and the globulin precipitate was brought into solution according to the directions of Weissman *et al.* (*8*). Following strictly the directions for those procedures, we found values of the albumin-globulin ratios that were lower than those reported by other investigators (*4,7,8*). An explanation for this discrepancy cannot be given; it may be stated that our fractionation data were perfectly reproducible.

The nitrogen content of the sera and of the protein fractions was determined by a modified Kjeldahl method combined with the amperometric titration of ammonia with hypobromite as reagent (*6*). No correction was made for nonprotein nitrogen.

Sulfhydryl determinations were made by amperometric mercurimetric titration at the rotated platinum-wire electrode as indicator electrode (*9*).

The disulfide content of whole serum and of its fractions was determined after partial acid hydrolysis of the proteins by amperometric mercurimetric titration at the dropping mercury electrode as indicator electrode (*5*), and also by argentometric titration at the rotated platinum-wire electrode. The argentometric titration previously developed for the determination of cysteine and cystine in an ammoniacal medium (*10*) has been modified for the present determinations, in which it was found that a strongly alkaline, nonammoniacal medium (0.25 *M* in sodium hydroxide, 0.4 *M* in potassium chloride, and 0.2 *M* in sodium sulfite) gave results in good agreement with those obtained in mercurimetric titrations.

Most of the disulfide determinations were carried out by the rapid argentometric titration technique, while the mercurimetric method served as a standard method for a check in doubtful cases. The data given for the disulfide content were not corrected for sulfhydryl and thus actually give the sum of disulfide and sulfhydryl, the latter being much smaller than the former.

Determination of Reactivity of Disulfide Groups with Sulfite

In the course of our studies on argentometric sulfhydryl titrations of proteins, an attempt was made to remove oxygen from the alkaline titration mixture with sodium sulfite—a simple artifice that is often

used in polarographic work. In fact, titrations of commercial crystalline bovine plasma albumin in aqueous or alcoholic (10 to 30% CH_3OH) ammoniacal medium, at *p*H not higher than 10.2, and 0.05 to 0.1 *M* in sodium sulfite, gave perfectly reproducible and correct results (0.66 mole SH per mole albumin). However, the results were not satisfactory when blood sera were titrated under the same conditions in the presence of sulfite. The galvanometer readings were unstable; well defined and reproducible end points could not be obtained; and the results were found to depend greatly on the temperature and on the rate at which silver nitrate was added to the mixture—higher results being obtained on the slow addition of the titrating agent. The results also varied with the composition of the buffer. An increase in the sulfite concentration or the presence of alcohol gave high results. Relatively accurate and reproducible results in the presence of sulfite were obtained when the titration mixture was kept at low temperature (not higher that 5° C.).

These observations indicate that under these experimental conditions bovine albumin is attacked very slowly or not at all by sulfite, while under the same conditions blood sera react with sulfite at an appreciable rate to give sulfhydryl groups that are titratable with silver nitrate. Assuming that all the sulfhydryl groups in the serum proteins can be titrated with silver in the absence of sulfite, it appears that part of the disulfide groups of the blood proteins react according to the equation:

$$PSSP + SO_3^{=} \rightleftharpoons PS^{-} + PSSO_3^{-} \qquad (1),$$

(*P* denotes protein) a reaction that has been studied quantitatively for cystine and dithiodiglycolic acid in this laboratory (*11*).

In a study of the reactivity of disulfide with sulfite in serum proteins from normal persons and from cancer patients, a procedure was developed in which the sulfite, after a given reaction time, was removed by precipitation as barium sulfite. The sulfhydryl formed from the disulfide could then be titrated with silver without further formation of sulfhydryl, and the results were reproducible. In contrast to cysteine, the establishment of the equilibrium in reaction (1) from right to left seems to be very slow with proteins. At the *p*H of the titration mixture used in the following procedure, no hydrolytic splitting of disulfide groups of the sera was detected during the time of the experiments.

Procedure

Eighteen ml. of an ammonia buffer (0.5 *M* in ammonia, 0.05 *M* in ammonium nitrate, *p*H=10.2) were introduced into a titration cell which was kept in a thermostat at 25° C. The cell was provided with a rubber stopper with holes for agar bridge, rotated platinum-wire electrode, buret, and inlet tube for nitrogen. The solution was made air-free with nitrogen which was passed through during the entire titration. To the air-free solution at a temperature of 25° C. was added 2 ml. of 0.1 *M* sodium sulfite solution and 0.5 ml. of serum. The time was noted when the serum was added. After the desired time of action of the sulfite, 1 ml. of a

saturated barium nitrate solution was added and the mixture titrated with a 10^{-3} *M* silver nitrate solution at an applied potential of −0.3 volt against the saturated calomel electrode.

Blank experiments with known amounts of cysteine and reduced glutathione showed that the barium sulfite did not coprecipitate sulfhydryl and did not interfere with the sulfhydryl titration. Stable galvanometer readings and well defined end points were obtained. The end point of this titration corresponded to the sum of reactive disulfide and of the sulfhydryl originally in the unreacted serum. The original sulfhydryl was determined in a separate titration of the serum in the absence of sulfite.

Expression of the Results

In the following table and figures the nitrogen content is expressed as grams nitrogen per 100 ml. of serum for the whole serum as well as for the albumin and globulin fractions. Sulfhydryl and disulfide content are given as micromoles SH per 100 ml. of serum and millimoles SS per 100 ml. of serum respectively, and also (in the graphs) as mg. cysteine and mg. cystine per 100 ml. of serum. Sulfhydryl and disulfide content are also expressed in moles SH and moles SS per mole protein for both albumin and globulin, taking 70,000 and 170,000 as molecular weights of albumin and globulin, respectively.

Results and Discussion

Nitrogen, Sulfhydryl, and Disulfide

It was found that the total nitrogen, sulfhydryl, and disulfide content of a given serum was equal to the sum of the corresponding values of its albumin and globulin fractions. Therefore most of the data for globulin were calculated from the data for whole serum and for the albumin fraction.

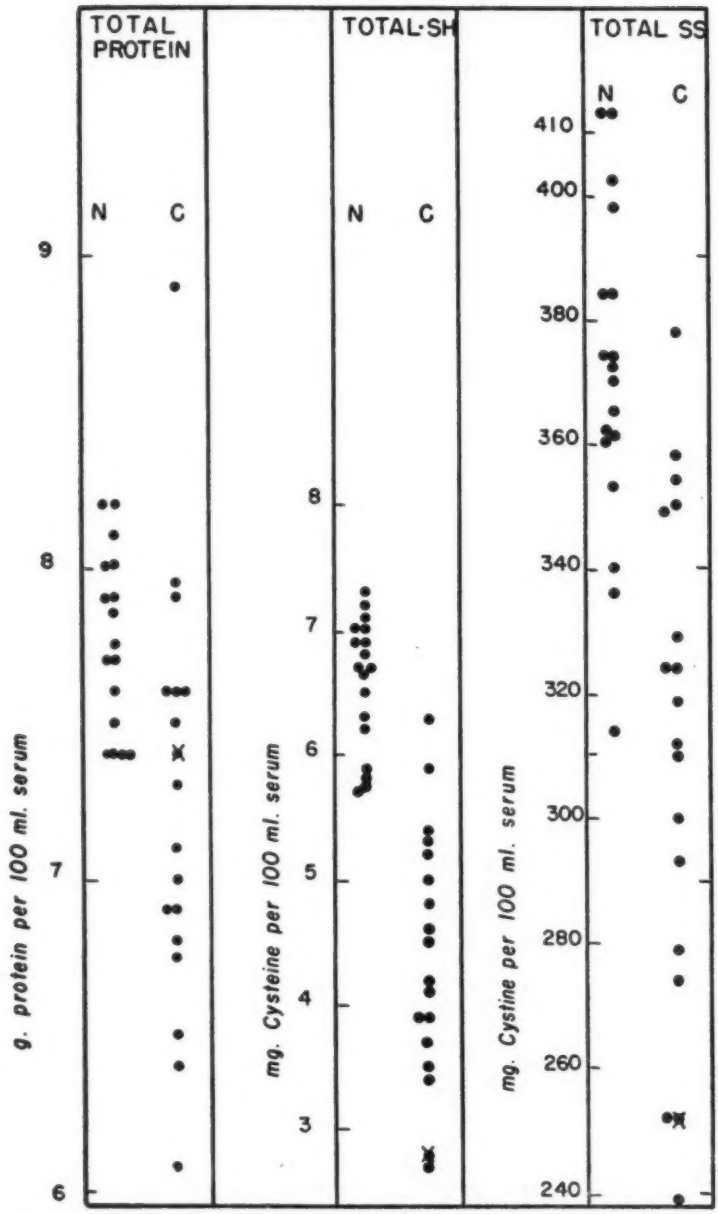
The data obtained with sera from 18 normal persons and from 17 cancer patients and 1 noncancer patient are given graphically in text-figures 1 and 2. Data from the pathologic sera are listed in table 1.

The lowest value of total protein in normal serum was 7.4 grams per 100 ml. of serum, while 59 percent of the cancer sera had values lower than 7.4 grams. In one patient (cancer of rectum) the nitrogen content of the serum was abnormally high. The fluctuation in total protein content was much greater in cancer sera than in normal sera. In agreement with Schoenbach *et al.* (*4*), the albumin nitrogen of cancer sera was found to be lower than that of normal sera, with the exception of three samples having albumin nitrogen values slightly above the lowest value found in normal sera (text-fig. 2). Although the albumin-globulin ratios reported by Schoenbach *et al.* differ from our values, the relative change of these ratios for normal and cancer sera is in reasonable agreement with our data. Thus these authors gave albumin-globulin ratios of 1.54 and 0.88 while our average values were 1.10 and 0.77 for normal and cancer sera, respectively.

TABLE 1.—*Nitrogen, sulfhydryl, and disulfide content of cancer sera*

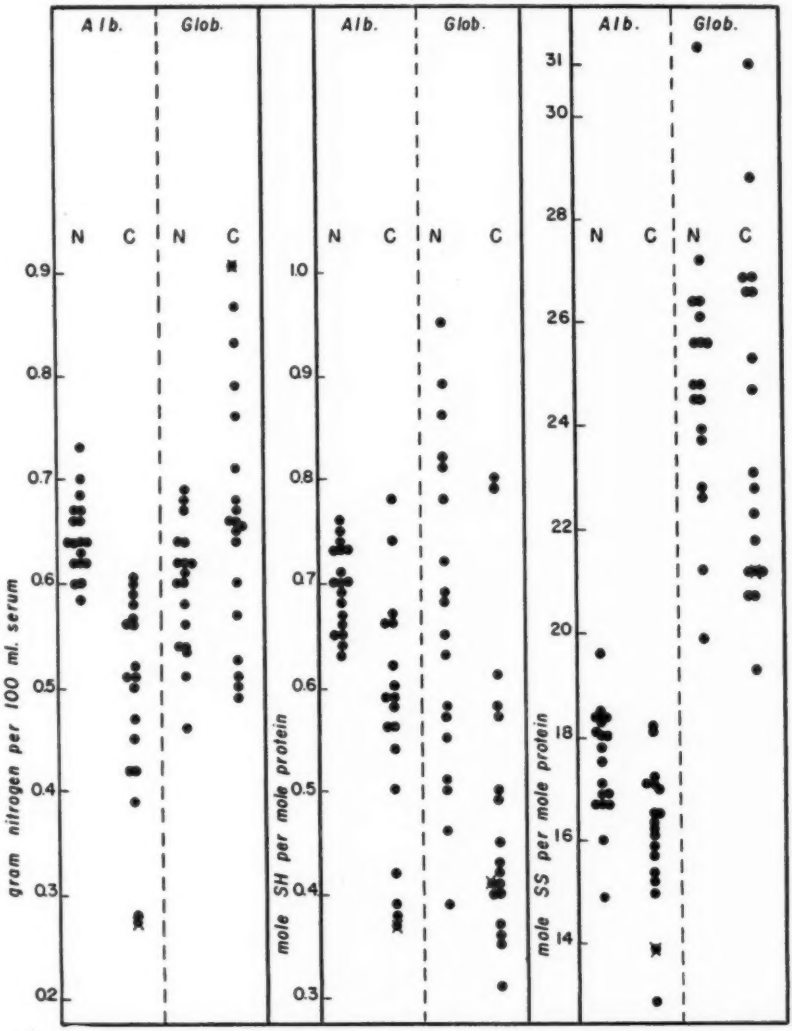
Clinical diagnosis	Nitrogen (*gm./100 ml. serum*)			Sulfhydryl (*μmole/100 ml. serum*)			Mole SH* per mole albumin	Mole SH† per mole globulin	Disulfide (*mmole/100 ml. serum*)			Mole SS per mole albumin	Mole SS per mole globulin
	Whole serum	Albumin	Globulin	Whole serum	Albumin	Globulin			Whole serum	Albumin	Globulin		
1. Ca. rectum	1.08	0.58	0.50	41.3	30.6	10.7	0.59	0.58	1.05	0.67	0.38	12.9	20.7
2. Ca. rectum	0.96	0.47	0.49	28.9	17.8	11.1	0.42	0.61	1.00	0.63	0.37	15.0	20.7
3. Ca. rectum	1.10	0.45	0.65	33.9	24.0	9.9	0.60	0.41	1.30	0.66	0.64	16.5	26.6
4. Ca. rectum	1.09	0.52	0.57	52.7	36.4	16.3	0.78	0.79	1.22	0.75	0.47	16.1	22.3
5. Ca. rectum	1.43	0.56	0.87	32.0	19.2	12.8	0.38	0.40	1.49	0.80	0.69	15.9	21.8
6. Ca. stomach	1.27	0.59	0.68	32.0	20.8	11.2	0.39	0.45	1.47	0.90	0.57	17.1	22.8
7. Bronchogenic Ca	1.17	0.51	0.66	40.0	26.2	13.8	0.58	0.57	1.35	0.75	0.60	16.5	24.7
8. Ca. an. rectum	1.22	0.56	0.66	43.3	33.1	10.2	0.66	0.42	1.46	0.82	0.64	16.2	26.6
9. Ca. esophagus	1.02	0.51	0.51	45.0	30.0	15.0	0.66	0.80	1.14	0.78	0.36	17.1	19.3
10. Ca. chest	1.26	0.61	0.65	43.5	31.7	11.8	0.59	0.49	1.33	0.82	0.51	15.2	21.2
11. Ca. rectum	1.22	0.39	0.83	30.7	19.4	11.3	0.56	0.37	1.37	0.60	0.77	17.2	25.3
12. Ca. neck, metastasis	1.10	0.50	0.60	38.0	30.0	8.0	0.67	0.36	1.45	0.82	0.63	18.2	28.8
13 to 17. Diagnosis not available	1.21—	0.60—	0.52—	48.8—	37.1—	11.7—	0.74—	0.50—	1.57—	0.97—	0.50—	15.4—	21.2—
	1.04	0.28	0.79	22.5	13.9	8.3	0.50	0.31	1.16	0.41	0.75	18.1	31.0
Duod. ulcer, diabetes	1.18	0.27	0.91	22.8	9.2	13.6	0.37	0.41	1.05	0.34	0.71	13.9	21.2

*Molecular weight of albumin taken as 70,000, nitrogen content 16%.
†Molecular weight of globulin taken as 170,000, nitrogen content 16%.



TEXT-FIGURE 1.—Graphs of total nitrogen, sulfhydryl, and disulfide contents of unfractionated normal (*N*) and cancer (*C*) sera. (×, noncancer pathologic serum.)

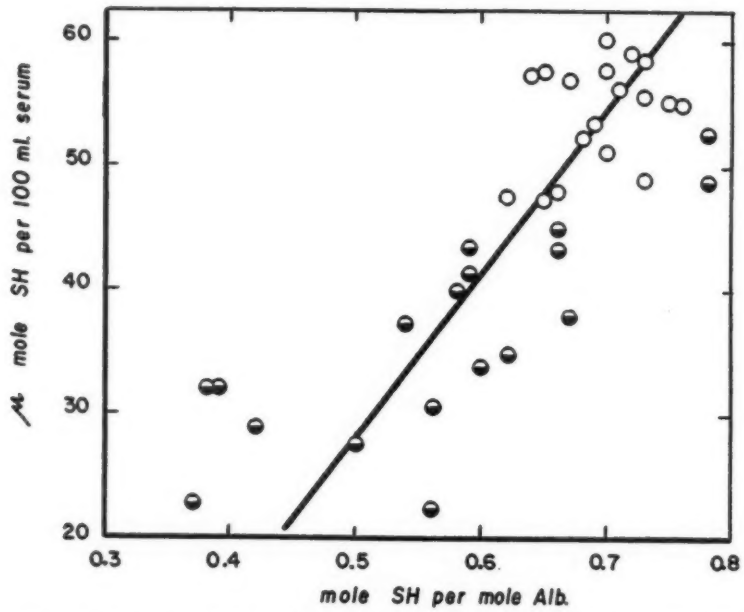
Our average value for albumin sulfhydryl in normal sera (0.69 mole SH per mole albumin) was in good agreement with values (0.71 and 0.67)



TEXT-FIGURE 2.—Graphs of nitrogen, sulfhydryl, and disulfide contents of albumin and globulin fractions of normal (*N*) and cancer (*C*) sera. (×, noncancer pathologic serum.)

reported by other authors (*8*, *12*). The variation in the albumin sulfhydryl of normal sera was relatively small (between 0.63 and 0.76 mole SH per mole albumin), but was much larger in cancer sera (0.38 to 0.78). In 12 of the 17 sera from cancer patients the sulfhydryl content was below the lowest value found in normal sera; 4 of the cancer sera had albumin sulfhydryl values within the normal range, and 1 (cancer of rectum, one day after operation) was found to have a higher albumin sulfhydryl content than the largest value observed in normal sera.

The data in table 1 and text-figure 2 also indicate that the low sulfhydryl content of cancer sera is mainly accounted for by the lower albumin content (and the lower albumin sulfhydryl), and to a much smaller extent by variations in the globulin fraction. Text-figure 3 presents graphically the total sulfhydryl content of normal and cancer sera in relation to the sulfhydryl content of the albumin fraction, and indicates an approximate proportionality between these two quantities.



TEXT-FIGURE 3.—μmole SH in 100 ml. serum versus mole SH per mole albumin for normal (○) and cancer sera (◒).

The sulfhydryl content of the globulin fraction as obtained by the method of Pillemer and Hutchinson (7) varied within rather wide limits in both normal and pathologic sera, and considerable overlapping between normal and pathologic values was observed. The sulfhydryl content of globulin (expressed as mole SH per mole globulin) ranged from 0.39 to 0.95 for normal sera and from 0.31 to 0.80 for cancer sera. Thus the globulin sulfhydryl offers a poor index for a differentiation between normal and pathologic sera. Our sulfhydryl determinations both in normal and cancer sera were in good agreement with those reported by Schoenbach *et al.* (4). Expressing the results in micromoles SH per gram nitrogen, these authors obtained, for instance, average values for normal sera of 44.3, 64.0, and 23.2 while our corresponding values were 43.9, 62.0, and 24.6 for whole serum, albumin, and globulin fractions, respectively. It is questionable whether the method of Pillemer and Hutchinson yields a reliable separation between the albumin and globulin fractions in both

normal and cancer sera. Until this is proved, no definite statement can be made on the basis of the sulfhydryl determination that there is a qualitative difference between albumin in the two kinds of sera.

The total disulfide content of normal sera, expressed as mg. of cystine per 100 ml. of serum, was found to be in the range from 314 to 413 (average 371), while the disulfide in cancer sera varied from 239 to 378 with an average of 311. Milone and Sullivan (*13*) reported normal disulfide values from 320 to 440 mg. (average from 38 determinations, 363 mg.), and values from noncancer pathologic sera from 225 to 375 mg. of cystine per 100 ml. of serum with an average value of 317. Thus the total disulfide content is somewhat reduced in most pathologic sera. The same was also found for the albumin disulfide, with an average value for cancer serum of 15.6 as compared with the average value in normal serum of 17.5 moles disulfide per mole albumin. No marked differences were found in the globulin disulfide of normal and cancer sera (average values 24.8 and 24.0 respectively).

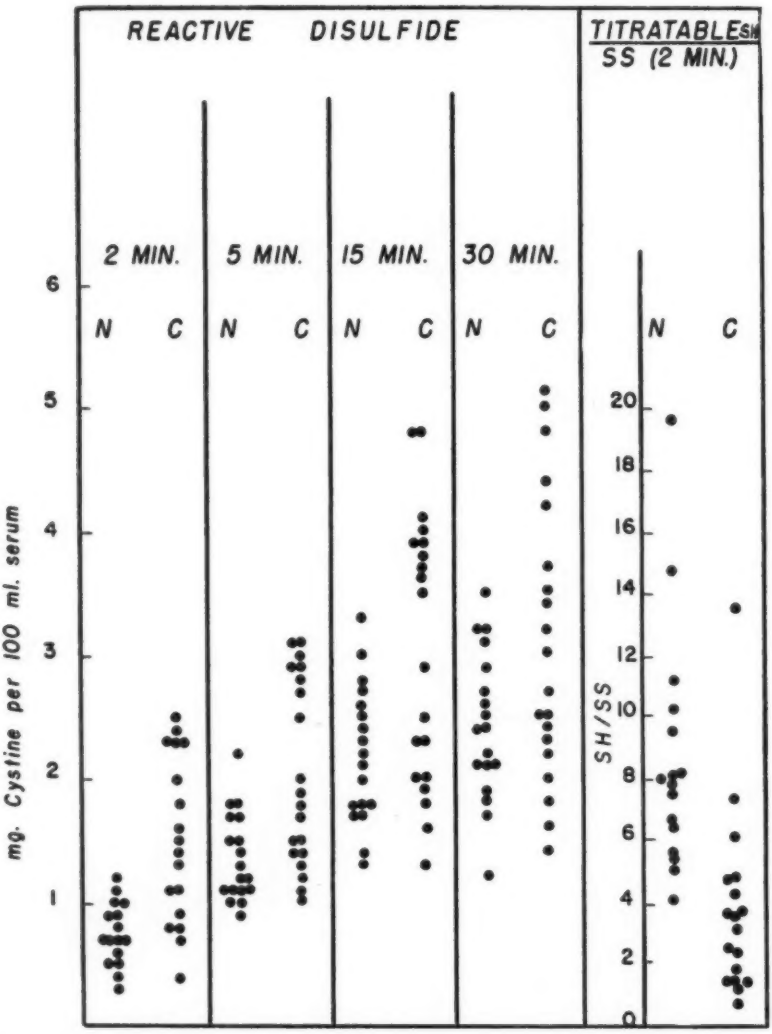
It would appear of interest to compare the content of other constituents in the albumin fraction of normal and of cancer sera. Results of such studies might allow an interpretation of the experimental fact that, in general, the disulfide content of albumin in normal sera is somewhat greater than in cancer sera.

Nitrogen, sulfhydryl, and disulfide values obtained in a pathologic noncancer serum (ulcer of duodenum, diabetes) are marked by a cross in text-figures 1 and 2. It is of interest to note that the data obtained with this serum deviate from the normal values to a larger extent than most of the cancer values do. A systematic study of noncancer pathologic sera would be desirable.

Disulfide Reactive with Sulfite

Sera from 20 cancer patients and 20 normal persons were allowed to react with sulfite for various periods of time (2, 5, 15, and 30 minutes), and the reacted disulfide was determined argentometrically after removal of the excess sulfite. Titration results are presented graphically in text-figure 4, in which the amount of disulfide that reacted after a given time is expressed as mg. of cystine per 100 ml. of serum.

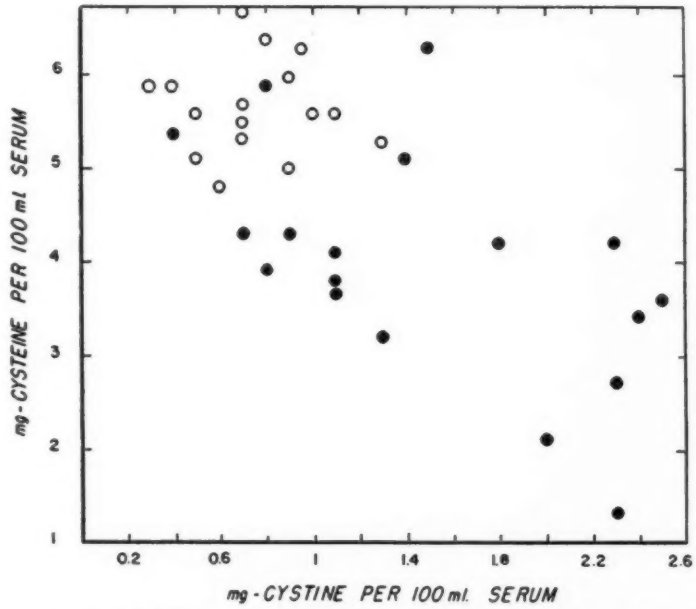
With almost all of the sera investigated, the amount of sulfhydryl formed increased with time during the first 15 minutes but remained constant or increased only slightly after prolonged action of sulfite (up to one hour). The maximum amount of disulfide reacting with sulfite under the experimental conditions was small compared with the total disulfide content in the serum. Thus in normal sera the amount of disulfide that had reacted after 15 minutes' action on sulfite varied from 1.4 to 3.3 mg. of cystine per 100 ml. of serum, while the average total amount of disulfide in these sera was 371 mg. of cystine per 100 ml. of serum. Comparing normal and cancer sera, it is seen from text-figure 4 that the differentiation was obtained during the initial period of reaction (after 2 minutes). At this stage 61 percent of the cancer sera gave a reactive disulfide content that was larger than the largest value in normal sera.



TEXT-FIGURE 4.—Disulfide reactive with sulfite in normal (*N*) and cancer (*C*) sera after various periods of time (2, 5, 15, and 30 minutes). Ratio of titratable sulfhydryl to reactive disulfide.

After 30 minutes of reaction only 3 percent of the cancer sera gave a reactive disulfide content larger than the maximum value in normal sera. It is also seen that the extent of overlapping of values for normal and for cancer sera increased with prolonged action of sulfite. The variation in the cancer values was generally larger than that in the normal values. The last column of text-figure 4 presents the relation between the total sulfhydryl content (mg. cysteine per 100 ml. serum) determined in the

absence of sulfite, and the reacted disulfide (mg. cystine per 100 ml. serum) determined after 2 minutes' action of sulfite. Although the variation in the values is large, it is seen that there were only six overlapping cases between normal and cancer values, with average SH/SS values of 6.0 and 3.5 for normal and cancer sera, respectively. Text-figure 5, which plots sulfhydryl against reactive disulfide, illustrates the trend for a large sulfhydryl content to be associated with a small amount of reactive disulfide. Preliminary experiments indicated that the source of reactive disulfide was mainly the globulin component of the serum.



TEXT-FIGURE 5.—Sulfhydryl versus reactive disulfide (after 2 minutes' action of sulfite). ○, normal sera; ●, cancer sera.

Further studies are planned which should show whether the difference between reactive disulfide in cancer and normal sera is accounted for by the difference in albumin-globulin content or by a difference in reactivity of these components in normal and cancer sera. An explanation for the fact that only a very small fraction of the disulfide groups of the serum proteins was accessible to reaction with sulfite, and that this fraction seemed to be related to the titratable sulfhydryl content of the protein, cannot be given at the present time. It is planned to investigate the action of sulfite on serum proteins in further detail, in order to make sure that the sulfhydryl in the reactive disulfide test actually originates with disulfide and is not derived from masked sulfhydryl in the native protein.

Summary

Nitrogen, sulfhydryl, and disulfide contents of blood sera and of their albumin and globulin fractions have been determined in sera from 18 normal individuals and from 17 patients with malignant tumors.

In agreement with other reports in the literature, the amount of albumin was found to be reduced in most of the pathologic sera, while the amount of globulin was normal or increased. The low sulfhydryl content of most cancer sera was due, in part, to a lower-than-normal molar sulfhydryl content of the albumin. The amount of sulfhydryl groups in the globulin fraction varied within relatively large limits in both normal and pathologic sera, and the globulin sulfhydryl is therefore not a promising index for a differentiation between normal and cancer sera. The total disulfide content of cancer sera was found to be less than normal in 44 percent of the cases investigated. In many of the cancer sera the molar disulfide content of the albumin was somewhat lower than in most of the normal sera (average 15.6 in cancer as compared with 17.5 in normal).

Sulfite did not react, or reacted only very slowly, with disulfide groups in albumin in an ammonia buffer (*p*H 10.2). A simple procedure is given for the determination of the reaction rate between sulfite and disulfide groups in proteins contained in blood sera. In 60 percent of the cancer sera the initial rate of this reaction was found to be larger than that in the normal sera.

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Demonstration of Protection Against the Lethal Toxicity of 6-Mercaptopurine in Mice[1]

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It has been demonstrated that 6-mercaptopurine acts as a purine antagonist for *Lactobacillus casei* (*1*, *2*). Its inhibitory activity for this microorganism was blocked competitively by adenine, guanine, xanthine, or hypoxanthine. The antagonist has been shown also to have an inhibitory effect on the growth of several transplantable tumors in mice (*3–5*), and has been employed in the treatment of patients with neoplastic disease (*6*, *7*). However, as with the other known antineoplastic agents, the toxicity of the drug to the host is one of the limiting factors in the use of 6-mercaptopurine (*4*). With the folic-acid antagonist aminopterin, enhancement of antileukemic effect was observed when the host-toxicity of the antagonist was reduced by citrovorum factor, by employment of appropriate temporal relationships of administration of metabolite and antagonist (*8*, *9*). In view of these results, it was considered desirable to determine whether the host-toxicity of 6-mercaptopurine could be similarly reduced. Successful control of toxicity could, presumably, afford greater opportunity to obtain enhanced antitumor specificity of action of 6-mercaptopurine, as well as of related purine antagonists.

Methods

The experiments were conducted in CDBA hybrid male mice (BALB/cAn $\times$ DBA/2J)F_1, 8 to 10 weeks old. The 6-mercaptopurine[3] was dissolved in dilute NaOH and titrated back to *p*H 8 to 10 with dilute HCl. The yeast adenylic acid, 2′-adenylic acid, 3′-adenylic acid, and 5′-adenylic acid were dissolved in dilute NaOH and injected at *p*H 6. Guanylic acid was partially dissolved in dilute NaOH and injected at *p*H 6 to 8. In one experiment, adenine, guanine, xanthine, and hypoxanthine were employed

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[2] National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

[3] 6-Mercaptopurine was kindly provided by Dr. George Hitchings of the Wellcome Research Laboratories, Tuckahoe 7, New York. Yeast adenylic acid (mixture of 2′- and 3′-adenylic acid), 2′-adenylic acid, 3′-adenylic acid, guanylic acid, and adenosine were from Schwarz Laboratories. 5′-Adenylic acid (My-B-Den) was from Ernst Bischoff Co. Guanine and hypoxanthine were from Bios Laboratories. Adenine, xanthine, guanosine, cytidylic acid and uridylic acid were from the Nutritional Biochemical Company. Diphosphopyridine nucleotide (cozymase "90") was from Sigma Chemical Company.

in aqueous suspension (table 4, expt. 1). In a second experiment the free purines were suspended in 5 percent acacia (table 4, expt. 2).

Guanosine was dissolved in dilute NaOH, brought back to *p*H 10 with dilute HCl, and injected as a partially soluble suspension. Adenosine was dissolved in dilute HCl and titrated up to *p*H 8 with dilute NaOH. Diphosphopyridine nucleotide was dissolved in distilled water and brought to *p*H 8 with dilute NaOH. Cytidylic acid and uridylic acid were dissolved in dilute NaOH and injected at *p*H 6 to 7. For compounds suspended in acacia, each dose was administered to the mice in the constant volume of 0.01 ml. per gm. of body weight. All other compounds were administered in the constant volume of 0.02 ml. per gm. 6-Mercaptopurine, guanosine, and the free purines suspended in acacia were injected subcutaneously. All other compounds, including the free purines in aqueous suspension, were injected intraperitoneally. The mice were maintained on an *ad libitum* diet of Purina laboratory chow and water, and were kept at a temperature of 78 to 80° F. The animals were weighed and observed daily. The individual experiments were terminated at 10 to 14 days following administration of the drugs, at which time all surviving mice were healthy in appearance and were equal to or had surpassed their initial weight.

Results

The results are summarized in tables 1 to 4. The two purine ribotides, guanylic acid and adenylic acid, afforded good protection against lethal toxicity when administered one hour prior to, or concomitantly with, 6-mercaptopurine. A high dose of guanylic acid (1,500 mg./kg.) administered one hour prior to 6-mercaptopurine resulted in a decrease in the mortality response at a series of dose levels of 6-mercaptopurine (table 1). Administration of guanylic acid itself resulted in some lethal toxicity in this experiment. On simultaneous administration, a relatively low dose of adenylic acid (125 mg./kg.) afforded good protection against a dose of 6-mercaptopurine that was consistently lethal for most of the animals (table 2). Although adenylic acid protected when administered one hour subsequent to 6-mercaptopurine, with either adenylic acid or guanylic acid loss in protection was evident when the drugs were withheld for 2 or 4 hours (table 2).

The other compounds tested were administered one hour prior to a dose of 500 mg. per kg. of 6-mercaptopurine (tables 3 and 4). 2′-Adenylic acid, 3′-adenylic acid, 5′-adenylic acid, diphosphopyridine nucleotide, adenosine, and guanosine protected against 6-mercaptopurine toxicity (table 3). The free purines adenine, guanine, xanthine, and hypoxanthine (table 4) did not appear to be as effective as the purine ribotides or ribosides. The two pyrimidine ribotides, uridylic acid and cytidylic acid, did not afford protection against the toxicity of 6-mercaptopurine (table 3).

TABLE 1.—*Effect of prior administration of guanylic acid on the lethal toxicity of 6-mercaptopurine**

Guanylic acid (*mg./kg.*)	6-Mercaptopurine (*mg./kg.*)	Mortality (*dead/total*)
	833	10/10
	500	9/10
	300	9/10
	180	6/10
	108	0/10
	64. 8	0/10
1500	833	6/10
1500	500	0/10
1500	300	1/10
1500	180	3/10
1500	108	1/10
1500	64. 8	0/10
1500	0	2/10

*Guanylic acid was administered one hour prior to the 6-mercaptopurine.

TABLE 2.—*The effect of time of administration of purine ribotides on the protection afforded against the lethal toxicity of 6-mercaptopurine*

Dose (*mg./kg.*)			Mortality (*dead/total*)			
Adenylic Acid (yeast):	Time (*hours*)*	6-Mercaptopurine	Expt. 1	Expt. 2	Expt. 3	Expt. 4
1000	−1	500	0/5	0/6	0/7	.....
1000	0	500	0/6	0/6	0/7	.....
500	0	500	.....	.....	.....	0/6
250	0	500	.....	.....	.....	0/6
125	0	500	.....	.....	.....	1/6
1000	+1	500	.....	0/6	.....	.....
1000	+2	500	.....	.....	3/7	.....
1000	+4	500	.....	.....	6/7	.....
1000	.....	0	0/12	0/18	0/20	0/6
500	.....	0	.....	.....	.....	0/6
Guanylic Acid:						
1500	−1	500	0/6	.....	.....	.....
1500	0	500	2/6	.....	.....	.....
1500	.....	0	0/12	.....	.....	.....
1000	−1	500	.....	.....	0/7	.....
1000	0	500	.....	.....	0/7	.....
1000	+2	500	.....	.....	7/7	.....
1000	+4	500	.....	.....	5/7	.....
1000	.....	0	.....	.....	0/20	.....
	.....	500	12/12†	4/6	9/10	7/8

*Time of administration of purine ribotides with respect to 6-mercaptopurine.

†6 of these mice received 0.02 ml. of distilled water per gm. of body weight, intraperitoneally, one hour prior to the 6-mercaptopurine.

TABLE 3.—*Effect of prior administration of purine ribotides, purine ribosides, and pyrimidine ribotides on the lethal toxicity of 6-mercaptopurine**

Dose (*mg./kg.*)		Mortality (*dead/total*)		
	6-Mercapto-purine	Expt. 1	Expt. 2	Expt. 3
Adenylic acid (yeast):				
1000	500	0/6	0/7	
1000	0	0/6	0/6	
2′-Adenylic acid:				
1000	500	0/6		
1000	0	0/6		
3′-Adenylic acid:				
1000	500	0/6		
1000	0	0/6		
5′-Adenylic acid:				
1000	500	0/6		
1000	0	0/6		
Adenosine:				
1000	500	.....	0/7	
1000	0	.....	0/6	
Guanosine:				
1000	500	.....	0/6	
1000	0	.....	0/6	
Diphosphopyridine nucleotide:				
1500	500	.....	0/6	
1500	0	.....	0/6	
1000	500	.....	2/6	
1000	0	.....	0/3	
Uridylic acid:				
1000	500	.....	.....	6/6
1000	0	.....	.....	0/6
Cytidylic acid:				
1000	500	.....	.....	5/6
1000	0	.....	.....	0/6
	500	5/6	6/6	4/6

*The purine ribotides, purine ribosides, diphosphopyridine nucleotide, and pyrimidine ribotides were administered one hour prior to the 6-mercaptopurine.

The course of fatal intoxication was similar to that described by Clarke *et al.* (*4*). In the current experiments most deaths occurred from 1 to 5 days following administration of 6-mercaptopurine. Occasional deaths were noted up to 10 days following treatment. The loss in body weight generally observed with lethal doses was reduced when the lethal toxicity was overcome by metabolite.

Discussion

These experiments demonstrate that the lethal toxicity of 6-mercaptopurine can be blocked in the mouse. The mechanism underlying this protection is under investigation. The competitive relationship demonstrated between purines and 6-mercaptopurine for *Lactobacillus casei* (*1, 2*) suggests that the same type of relationship may obtain for the whole animal. No attempt was made in this study to determine the relative protection afforded by the compounds active against 6-mercaptopurine. The data indicate that the purine ribotides and ribosides were more

TABLE 4.—*The effect of prior administration of free purines on the lethal toxicity of 6-mercaptopurine**

Dose (*mg./kg.*)		Mortality (*dead/total*)	
	6-Mercapto-purine	Expt. 1	Expt. 2
Adenine:			
1000	500	4/5	4/6
1000	0	3/6	3/6
500	500	.....	1/6
500	0	.....	3/6
Guanine:			
1000	500	3/6	2/6
1000	0	0/6	0/6
500	500	.....	5/6
500	0	.....	0/6
Xanthine:			
1000	500	6/6	5/6
1000	0	0/3	0/6
500	500	.....	4/6
500	0	.....	0/6
Hypoxanthine:			
1000	500	3/6	4/6
1000	0	1/5	0/6
500	500	.....	4/6
500	0	.....	0/6
	500	5/6	8/10
Acacia controls.....	.....	.....	0/6

*The free purines were administered one hour prior to the 6-mercaptopurine.

effective than the free purines. This may mean that the purines must be converted to ribotides or ribosides to protect successfully against 6-mercaptopurine. However, it is possible that this difference may be caused by the relative insolubility or greater toxicity of the purines. The activity of diphosphopyridine nucleotide may be attributable to the adenosine moiety. That the pyrimidine ribotides afforded no protection against 6-mercaptopurine indicates that the protection may be specific for purine derivatives. The loss of protection against 6-mercaptopurine on delayed administration of high doses of adenylic acid or guanylic acid indicates that the action of 6-mercaptopurine in the host is rapid, and relatively irreversible with respect to these ribotides.

Several examples have been provided illustrating how the relative effect of a drug on tumor and host, defined as the specificity of action of a drug, may be altered by administration of a second drug (*8–10*). Administration of citrovorum factor concomitantly with aminopterin, or folic acid one hour prior to aminopterin, diminished the antileukemic specificity of action of aminopterin, by protecting the tumor relatively more extensively than the host. Administration of citrovorum factor subsequent to aminopterin increased the antileukemic specificity of action of the aminopterin. It did this by protecting the host relatively more extensively than the tumor. The ability to protect against the toxicity of 6-mercaptopurine for the host will permit the utilization of methodology similar to that employed with aminopterin and citrovorum factor

(*9*, *10*) to determine to what extent the specificity of action of 6-mercaptopurine may be altered. Attempts to block the inhibitory effect of 6-mercaptopurine against tumor, employing free purines, folic acid, or leucovorin, have been relatively unsuccessful (*4*, *5*). If with the compounds that afford host-protection against 6-mercaptopurine more extensive protection should be afforded to the host than to the tumor, enhanced antitumor activity of 6-mercaptopurine could result. This possibility is now being investigated.

Summary

Protection was afforded against the lethal toxicity of 6-mercaptopurine in normal mice by the administration of purine ribotides, purine ribosides, and diphosphopyridine nucleotide. The free purines were not as effective in affording protection against the toxicity of the antagonist. The pyrimidine ribotides tested were ineffective in influencing toxicity.

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The Effect of Weight Reduction on the Occurrence of Spontaneous Mammary Tumors in Mice[1, 2]

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Waxler, Tabar, and Melcher recently reported that virgin C3H female mice made experimentally obese showed an earlier appearance and a greater incidence of spontaneous mammary carcinoma than did control mice of normal weights (*1*, *2*). Throughout this experiment these two groups of mice were allowed continuous free access to food. The animals that became obese consumed a greater amount of ration during the period of development and maintenance of this obesity. A correlation between tumor formation, obesity, and increased caloric consumption was apparent.

The present experiment was undertaken to determine the rate of appearance of mammary tumors in mice that had been reduced to normal weights following a period of obesity. The study consisted of three groups of mice: a control group, an obese group, and a previously obese group that was maintained in the same weight range as the controls by pair-feeding throughout the experiment.

Materials and Methods

Strain C3H mice, known to have a high incidence of mammary tumors, were used in this experiment. All the animals were females and they were kept in a virgin state throughout the entire experiment. Their exact ages were known. The animals were housed six to a cage and allowed free access to Purina chow and water up to the age of two months. At this time they were divided into three groups, of which two groups were given a toxic dose of gold thioglucose[3] (*2*). The survivors of this injection of gold were weighed, and set aside to be compared at intervals with the control group. Within one month, most of these treated mice had become obese. At this time, one group of the obese mice was placed in individual cages and starved down to a weight equal to that of the untreated controls. Henceforth these two groups (the previously obese and the controls) were kept at similar weights by pair-feeding. The obese group had con-

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[3] The gold thioglucose was generously supplied by Schering Corp., Bloomfield, N. J.

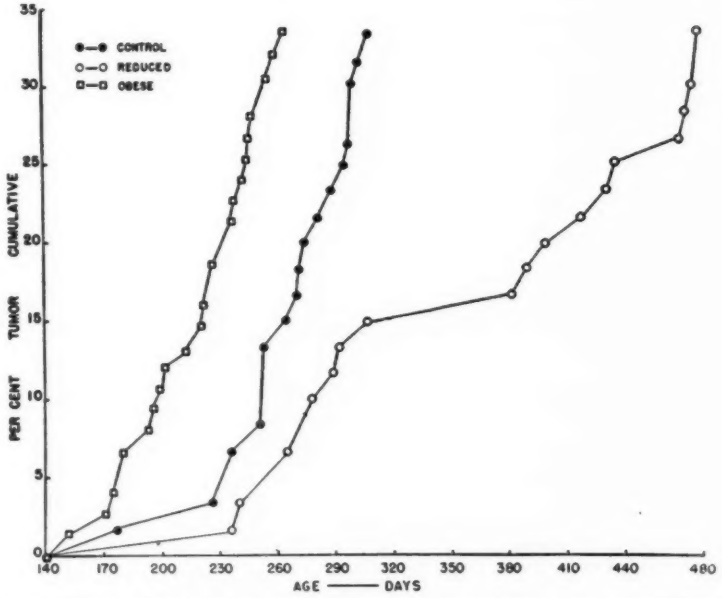
tinued free access to the chow throughout this time and to the end of the study. The mice were examined at intervals for spontaneous mammary tumors, and the survival time subsequent to the appearance of these tumors was noted. The experiment was discontinued when 33⅓ percent of the mice of each group showed tumors. The effective totals were 60 controls, 75 obese, and 60 reduced mice—the number of mice in each group when one third of that group had developed tumors.

Results

Table 1 lists the individual weights and the time of appearance of the tumors in each of the three groups of mice. The average weight of the previously obese mice that developed tumors was 29.1 grams and compared favorably with the average of their pair-fed, untreated controls, which was 28.5 grams. The mice in the obese group averaged 52.8 grams. The tabulated data show that the obese mice had their tumors by 265 days, whereas 307 days were needed by the controls and 480 days by the reduced mice. The average time of appearance of tumors in the three groups was as follows: obese, 219 days; control, 267 days; reduced mice, 365 days.

TABLE 1.—*Appearance of spontaneous mammary tumors in obese, previously obese, and control C3H mice*

Animal No.	Control (20 mice)			Obese (25 mice)			Previously obese (20 mice)		
	Age of tumor onset (days)	Survival after tumor onset (days)	Weight at tumor onset (gm.)	Age at tumor onset (days)	Survival after tumor onset (days)	Weight at tumor onset (gm.)	Age at tumor onset (days)	Survival after tumor onset (days)	Weight at tumor onset (gm.)
1	177	94	28	152	75	45	236	134	32
2	226	94	32	171	89	49	240	35	28
3	236	56	30	175	45	42	265	49	27
4	236	82	32	177	64	55	265	83	27
5	251	51	32	177	70	48	278	70	29
6	253	46	25	193	17	53	278	18	28
7	253	53	30	196	149	55	289	45	28
8	253	127	29	197	62	46	292	22	29
9	265	69	29	202	98	51	307	48	28
10	271	28	30	213	28	56	382	66	30
11	272	59	22	221	55	43	390	35	30
12	275	33	27	222	63	55	400	87	27
13	282	45	26	227	57	54	419	113	30
14	289	45	27	227	57	60	432	90	28
15	296	45	28	237	103	52	439	72	28
16	298	95	27	237	50	55	470	76	29
17	299	48	30	238	48	56	473	56	33
18	299	77	28	243	68	52	477	52	32
19	303	56	29	245	49	55	480	89	28
20	307	123	29	246	73	59	480	132	32
21				248	88	58			
22				256	76	59			
23				256	63	58			
24				260	37	48			
25				265	60	56			
Average	267	66	28.5	219	66	52.8	365	69	29.1



TEXT-FIGURE 1.—Incidence of spontaneous mammary carcinoma in virgin female C3H mice.

Text-figure 1 plots the ages of the three groups of mice at the time of appearance of the tumors against the cumulative percentage of animals developing mammary carcinoma. It is seen that at 265 days, when 33⅓ percent of the obese mice had tumors, only 15 percent of the control and 7 percent of the reduced mice were tumor-bearing.

Discussion

In an earlier experiment we compared the appearance of spontaneous tumors in two groups of C3H mice—in those made obese by injection of gold thioglucose and in control mice (*1*). These results noted that the occurrence of spontaneous tumors in the obese mice was significantly earlier. This present study repeated in part the earlier work and again emphasized similar findings. The results obtained from the previously obese, pair-fed animals required some interpretation. The incidence of tumors in this group was significantly less than that of their pair-fed partners and very much less than the obese group. Data not incorporated in this paper showed that the appearance of tumors in this reduced group maintained this slow rate. In fact, when most of the controls and other mice had died, a high percentage of reduced animals were still living and free of tumors.

We previously reported on the food requirements of such obese mice (*3*). These mice required an increased amount of food during the development and maintenance of this obesity. On starvation-refeeding experi-

ments these obese mice demonstrated an ability to mobilize their excess fat during starvation. Obese mice, starved down to their original weight, could regain their prestarvation weight on refeeding. Thus, mice made obese following gold thioglucose treatment appear to have a permanent trend toward obesity and a need for increased food intake to attain this heavier weight.

Several investigators have shown that animals on a restricted food intake show delay in spontaneously occurring and experimentally induced neoplasia (*4–6*).

The reduced pair-fed mice in this present experiment appeared to be on a diet lower in caloric value than that which they constitutionally require to reach their ultimate and potential size and weight. This may account for a lower tumor incidence than would be expected for mice of this weight range.

Although we noted the time of survival subsequent to the appearance of tumors in the three groups of mice, we have made no evaluation of these data. The averages are quite close, but the range in each group is marked. The factors associated with longevity following appearance of tumors were not taken into consideration in this particular experiment.

Summary

1) Virgin C3H female mice made experimentally obese develop spontaneous mammary tumors earlier than control mice of normal weights.

2) Mice made obese, and subsequently reduced and maintained at the weight of their controls by pair-feeding, show a slower rate of appearance of tumors than do these control mice.

3) The average time of appearance of tumors in the obese mice was 219 days, in the controls 267 days, and 365 days in the previously obese, reduced mice.

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Response of Patients with Advanced Neoplasms to the Intravenous Administration of *Alpha*-Peltatin [1, 2]

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In 1942, Kaplan (*2*) reported on the successful treatment of condylomata acuminata by the topical application of podophyllin. This was quickly confirmed by numerous investigators, and led to trial of podophyllin in the topical treatment of other clinical conditions. A short summary (*3*) of this subject, with a bibliography of 98 references, was recently published by Penick and Company. A comprehensive critical review of the chemical, biological, and clinical literature on podophyllin has been published by Kelly and Hartwell (*4*).

Sullivan (*5*) obtained regression of cutaneous carcinomas with podophyllin applied locally, but subsequently reported (*6*) recurrence within two years in all the patients.

In 1947, Belkin (*7*) and Hartwell and Shear (*8*) reported that subcutaneous administration of podophyllin affected transplanted tumors in mice, but effective doses were toxic. Fractionation of the resin from the American variety of May apple (*P. peltatum*) yielded three active substances. One of them was identified as podophyllotoxin (*8*), which had been isolated (*9*) in 1880. The other two substances (*10*), isolated from podophyllin for the first time, were named *alpha*-peltatin and *beta*-peltatin, respectively.

Each of these three highly potent components of podophyllin was administered (*11*) parenterally to mice bearing five types of transplanted tumors and to rats bearing a transplanted carcinoma. Extensive damage was induced in these diverse types of tumors following a single subcutaneous injection. Repeated injection of α-peltatin in animals bearing lymphatic tumors resulted in retardation of tumor growth and somewhat longer survival of the mice.

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[2] A preliminary report (*1*) was presented at the New York meeting of the American Association for Cancer Research, April 13, 1952.

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[6] National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

The effects of toxic doses of these three compounds were investigated (*12*) in normal laboratory animals of four species: mice, rats, rabbits, and dogs. The route of administration (intravenous, subcutaneous, or intramuscular) appeared to have little influence on the toxicity. The range in which a dose-response curve was observed was narrow. Toxic manifestations such as diarrhea, vomiting, prostration, and (in dogs) salivation usually appeared 1 to 4 hours after the injection.

The changes in peripheral blood observed (*12*) after near-lethal doses were: an initial leukopenia involving all cellular elements after ½ to 5 hours; a leukocytosis and a marked normoblastosis, accompanied by degenerative changes in the neutrophils, after 5 to 24 hours; a leukopenia after 24 to 72 hours; and a rapid recovery to the normal picture within 4 to 5 days following the injection.

Examination of the tissues after administration of a lethal or a maximum tolerated dose of α-peltatin showed degenerative cytologic alterations in the spleen, thymus, lymph nodes, and bone marrow as early as 3 to 6 hours after injection. Similar changes were seen in the testes, ovaries, and epithelium of the upper gastrointestinal tract. Definite histologic evidence of hepatic, renal, or cardiac damage was not seen, even after a lethal dose.

The tumor-damaging effect in animals bearing a carcinoma or a melanoma was obtained only with doses near the level toxic for non-tumor-bearing mice. On the other hand, tumor damage was induced in the lymphomas and in Sarcoma 37 with doses that were well below those producing toxic effects in normal mice.

In view of the striking changes induced in animal tumors following injection of the active components of podophyllin, investigation of the effect of α-peltatin administered intravenously in patients with advanced neoplasms was deemed warranted. This paper reports the responses observed in 45 patients with various types of neoplasms. Although evidence of therapeutic value was not obtained, α-peltatin did induce necrosis and shrinkage of neoplastic tissue in some of the patients.

Materials and Methods

A stock 1-percent solution of α-peltatin (*13*) was prepared, at monthly intervals, in acetone N.F. At first, each ml. of the stock solution was diluted with 0.1 ml. *N* NaOH and 0.9 ml. distilled water; the alkali was employed to make the drug water-soluble. In this alkaline solution, however, it is rapidly converted to an isomer (*13*) of much lower potency (*10*); hence the solution was administered promptly after preparation.

When the solution was injected intravenously by syringe, transient burning pain was felt along the course of the vein; phlebitis was observed after repeated injection. The use of alkali was thereupon discontinued. Instead, an aliquot of the stock solution in acetone was added to 200 ml. of a 10-percent solution of glucose in distilled water; this was given by intravenous infusion. During the 20 to 30 minutes required for adminis-

tration, the finely divided suspension of the drug did not sediment, and there were no accompanying untoward local reactions. The number of patients given the naturally occurring isomer was 45.

α-Peltatin-B, the dextrorotatory diastereoisomer obtained (*13*) on treatment with alkaline reagents, was given in a similar fashion to 5 patients. Aliquots up to 5 ml. (50 mg.) of a 1-percent solution in acetone were added to 250 ml. of the 10-percent aqueous glucose solution. Larger doses (150 to 250 mg.) were given in 250 ml. of plasma but the precipitation of the plasma, by the acetone in which the drug was dissolved, limited the dosage of this relatively inactive isomer.

Fifty patients with various types of metastatic neoplasms were selected for this study. The distribution of cases (leukemia, malignant lymphomas, multiple myeloma, and carcinoma) is listed in table 1. Most of the lesions were considered inoperable, or were recurrent after surgery, radiation, or conventional medical therapy. The diagnosis in each case was established by tissue biopsy; whenever feasible, serial biopsies were obtained [7] during the investigation.

Serial examinations were carried out to detect alteration in function of the liver, kidneys, heart, or central nervous system that might indicate drug toxicity or provide clues on the mechanism of action of the drug. These included repeated neurological and electrocardiographic examination, urinalysis, and the following chemical determinations on blood serum: urea nitrogen, total protein, albumin-globulin ratio, bilirubin, thymol turbidity, zinc sulfate turbidity, and alkaline phosphatase. In addition, the following special determinations were carried out: β-glucuronidase activity of serum and biopsied tissues (*14*); mucoprotein and polysaccharide level of serum (*15*); serum level and urinary excretion of uric acid (*16*, *17*); urinary excretion of reducing corticoids (*18*) and of 17-ketosteroids (*19*); eosinophil count in peripheral blood (*20*); and sulfhydryl content of serum proteins by amperometric titration (*21*).

Dosage.—The initial dose employed was 0.03 mg. per kg. of body weight. This was gradually increased to a maximum of 0.5 mg. per kg. without encountering serious untoward effects in the first few patients.

After these exploratory trials, treatment was instituted with dosage in the range 0.1 to 0.3 mg. per kg. Initially, repeated doses were given at intervals of 5 to 7 days; as experience was accumulated this interval was reduced to 1 to 3 days. The most intensive treatment in a single case consisted of 11 injections over a 22-day period with a total dose of 170 mg. (4 mg./kg.). Another patient received a total dose of 0.9 mg. per kg., given in 3 injections over a 4-day period.

The dosage was limited by appearance of transient signs of toxicity after a single dose in the range of 0.15 to 0.39 mg. per kg. When a dose that evoked signs of toxicity in a given patient was repeated two or three times weekly, intractable reactions usually developed which necessitated reduction of dose or interruption of treatment. Frequently, only a

[7] By Dr. John E. Wirth, Chief of the Tumor Service, and Dr. A. G. Morrow, of the U. S. Public Health Service Hospital in Baltimore.

slight lowering of the dose level was sufficient to give freedom from symptoms of toxicity.

Results

Forty-five patients, varying in age from 4 to 80 years and having a variety of disseminated neoplasms, were treated with α-peltatin. There were 18 patients with carcinoma (including 2 with melanocarcinoma) and 27 with lymphoma, leukemia, or myeloma. Five additional patients were given, in a similar fashion, the relatively inactive isomer α-peltatin-B. The pathologic diagnosis in the individual cases is indicated in table 1. With the possible exception of one patient with giant follicular lymphoma, no therapeutic effects were noted that were comparable to results produced by other substances available for the palliative treatment of neoplastic diseases. Development of objective changes in neoplastic tissue was observed in a number of instances (table 2) within 6 to 24 hours after administration of doses that produced acute febrile and gastrointestinal manifestations. In addition to these changes, many patients noted the acute onset of severe pain at the sites of neoplastic involvement. The pain, together with the febrile and gastrointestinal symptoms, usually subsided 12 to 24 hours after injection of the drug.

A. Lymphoid neoplasms.—Twenty-seven patients with various forms of leukemia, lymphoma, and myeloma received α-peltatin. Among the leukemia group there were four patients with acute leukemia and three with chronic myeloid leukemia. No evidence of significant improvement in the hematologic status was noted in these patients. Distinct recession in splenomegaly was observed in the three cases of chronic myeloid leukemia and in three of the four cases of acute leukemia within 24 hours after the larger doses of α-peltatin. Such doses induced fever and gastrointestinal symptoms in each instance. In one patient with acute leukemia, no effect on progressive splenomegaly was noted during treatment with several large doses which were given at widely spaced intervals.

Six patients with Hodgkin's disease received α-peltatin. Four of these were of the moderately advanced, disseminated, abdominal type with remittent fever, pancytopenia, hepatosplenomegaly, and retroperitoneal adenopathy. One patient had only a peripheral involvement and one patient was in a moribund state with massive abdominal peripheral and mediastinal involvement; in the latter, marked regression of lymph-node size and of a massively enlarged spleen occured (text-fig. 1) within 24 hours after the first dose (20 mg.) of α-peltatin. The reduction of lymphoid tissue in areas accessible to examination was estimated to be about fifty percent. The dose that induced these changes produced a shaking chill 2½ hours after injection, fever of 104° F., nausea and diarrhea. The diarrhea persisted during the subsequent two weeks, when four additional injections were given. The clinical course was not altered despite the obvious and rapid reduction in neoplastic tissue. Post-mortem examination showed Hodgkin's disease with diffuse necrosis of neoplastic tissue involving the retroperitoneal regions, omentum, liver, and spleen.

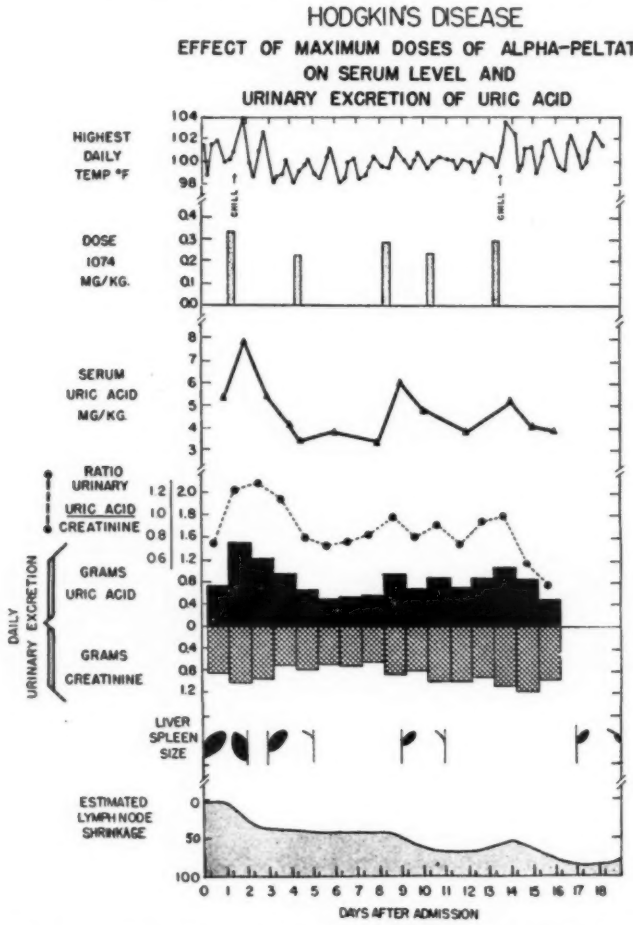
TABLE 1.—*Case distribution; intravenous administration of α-peltatin*

Drug	Type of neoplasm	Number of patients
α-Peltatin	*Lymphomas:*	
	Acute leukemia	4
	Chronic myeloid leukemia	3
	Chronic lymphatic leukemia	1
	Giant follicular lymphoma	2
	Lymphosarcoma	5
	Hodgkin's disease	6
	Recticulum-cell sarcoma	2
	Multiple myeloma	4
		27
	Carcinomas:	
	Adenocarcinoma	
	Breast	2
	Prostate	1
	Lung	2
	Unknown primary site	1
	Squamous-cell carcinoma	
	Ear	1
	Buccal mucosa	2
	Tongue	1
	Cervix	2
	Anorectum	1
	Melanocarcinoma	2
	Anaplastic carcinoma	
	Lung	2
	Unknown primary site	1
		18
α-Peltatin-B	Hodgkin's disease	1
	Lymphosarcoma	1
	Multiple myeloma	1
	Chronic myeloid leukemia	1
	Adenocarcinoma of lung	1
		5

TABLE 2.—*Objective changes in patients during administration of α-peltatin*

Patients		Changes observed	
Type	Number	Nature	Number of cases
Carcinoma	18	Diffuse tumor slough	1
		Focal tumor slough	2
		Lymph-node shrinkage	4
		No change	11
Lymphoma or leukemia	27	Shrinkage of spleen and/or lymph node	19
		No change	8
		Clinical remission	1*

*Giant follicular lymphoma.



TEXT-FIGURE 1.—Chart showing alterations in the blood level and urinary excretion of uric acid in a patient with massive Hodgkin's disease in the terminal phase. Within 24 hours after the first injection, the peripheral lymph nodes shrank to about half their previous size, and complete recession of massive splenomegaly was observed. No alteration of the clinical course was apparent despite these objective changes. The patient died five days after last injection of α-peltatin. Autopsy showed diffuse, necrotic lesions of Hodgkin's disease.

In three of the other four patients with abdominal Hodgkin's disease, a definite recession in palpable splenomegaly (*cf.* fig. 1) was observed within 24 hours after dosage with α-peltatin, which induced toxic manifestations. However, progressive increase in spleen size began again 3 to 4 days after the last injection had been given.

Partial diminution of lymph-node size in nonirradiated lesions was observed, after large doses of α-peltatin, in four of five patients with disseminated lymphosarcoma and in one patient with disseminated

reticulum-cell sarcoma. No effect was noted in one patient with reticulum-cell sarcoma of the neck, who had previously received intensive roentgen treatment. No significant amelioration of the clinical course in any of these patients with Hodgkin's disease, reticulum-cell sarcoma, or lymphosarcoma was observed, despite the local changes observed in the lymph nodes and spleen. An apparent alteration in clinical course was observed in one patient with giant follicular lymphoma. In this case there was reduction in size of spleen and lymph nodes, and subsidence of a pleural effusion, after treatment with α-peltatin. This remission lasted for nine months. A second temporary remission occurred after one injection of α-peltatin. In another patient with giant follicular lymphoma, the clinical course was not altered despite lymph-node shrinkage.

Four patients with multiple myeloma were given 5 to 11 injections of α-peltatin over a period of three to four weeks. The lowest tolerance to α-peltatin was observed among these patients with multiple myeloma. Febrile and gastrointestinal reactions occurred after doses that were only about one-third those required to induce these reactions in patients with carcinoma. Favorable changes were not observed in these patients.

B. Epithelial neoplasms.—Three patients with ulcerating, fungating squamous-cell carcinoma were treated with α-peltatin. The buccal mucosa was the primary site in two patients; the pinna of the ear in one. Two of the patients developed progressive necrosis and a black slough 6 to 24 hours after a single large dose, which produced moderately severe febrile and gastrointestinal symptoms, and pain in the lesions.

In the first of these patients (figs. 2 and 3) a diffuse slough was evident throughout a mass 8 × 10 cm. in size. Within 48 hours, the base of this lesion appeared grossly free of tumor. However, biopsies at the base showed the presence of small scattered islands of tumor cells from which regrowth occurred rapidly. A subsequent injection of α-peltatin also induced discoloration and diffuse necrosis in the base of the tumor. The margin of the tumor, however, continued to extend during the ensuing few weeks. In the second patient, with a massive tumor in the pharynx, necrosis developed in accessible satellite lesions in the cheek. Biopsies obtained 6 to 24 hours following treatment showed that the number of necrotic tumor cells was slightly increased, on comparison with a pretreatment biopsy. The third patient, with a comparable lesion, showed no changes after administration of α-peltatin.

Two patients with metastatic breast carcinoma received α-peltatin. In the first one a fungating, ulcerating, superficially infected adenocarcinoma of the breast flattened and became necrotic on the outer borders of the lesions. Microscopic examination of pretreatment and posttreatment biopsies from the breast lesion did not show changes greater than might have been expected from sampling variation alone. In the second patient an axillary lymph node removed 24 hours after the last of three large doses of α-peltatin showed diffuse necrosis of tumor cells. However, a control biopsy had not been available immediately prior to administration of the drug. In this latter patient no effect was noted on a massively

enlarged liver involved with neoplasm, despite persistent treatment with large doses.

In one patient with an adenocarcinoma of unknown primary site, a cervical lymph node removed prior to treatment showed spontaneous scattered areas of necrosis. An axillary lymph node removed 24 hours after the last of three large doses of α-peltatin showed diffuse karyorrhexis, pyknosis, and necrosis. These changes were associated with the gross observation of slight shrinkage and softening of the areas of lymphatic involvement.

Three patients with anaplastic carcinoma were given α-peltatin. Severe pain and shrinkage in lesions, with evidence of tumor necrosis, was observed in one of them. The lesions in this patient originated from the lung and were disseminated throughout the chest wall, with retrograde lymphangitic spread in the superficial tissues of one arm. The second patient, with anaplastic axillary metastases from a lesion of unknown origin, likewise developed acute pain and shrinkage of lesions after a large dose of α-peltatin. The third patient, with an anaplastic carcinoma of the lung, did not show objective evidence of change.

No gross or microscopic changes were observed in the following types of tumors treated with α-peltatin: carcinoma of the cervix previously treated with intensive local radiation; squamous-cell carcinoma of tongue; adenocarcinoma of the lung; squamous-cell carcinoma of the anorectal region; melanocarcinoma; and prostatic carcinoma.

In summary, 18 cases of carcinoma received from 1 to 11 injections of α-peltatin. No symptomatic relief or alteration in the patients' status was observed, despite some evidence of shrinkage of involved lymph nodes (4 cases) and development of induced tumor necrosis (gross in 1 case, focal in 2, and suggestive evidence microscopically in 6 cases). The evaluation of induced histologic changes was complicated by the presence of inflammatory changes and spontaneous necrosis in lesions, which vary in extent not only from tumor to tumor but also in different specimens taken from the same tumor.

C. Combination with other chemotherapeutic agents.—An attempt was made to determine whether administration of α-peltatin in conjunction with other chemotherapeutic agents would result in potentiation of tumor inhibition in man such as has been demonstrated in tumor-bearing animals (*22*).

Three patients with Hodgkin's disease were given α-peltatin during treatment with therapeutic doses of nitrogen mustard [methyl-bis(β-chloroethyl) amine hydrochloride]. The α-peltatin was administered in an intravenous drip prior to the nitrogen mustard. No interference with or enhancement of the fever and gastrointestinal symptoms induced by α-peltatin, or the posttreatment leukopenia induced by nitrogen mustard, was observed; nor was there any recognizable increase in the toxicity of either drug when employed in combination. The clinical results did not appear to be any more favorable than might have been expected with nitrogen mustard alone.

α-Peltatin was also administered during treatment with ACTH in four patients and with cortisone in one patient having Hodgkin's disease, without any definite evidence of a synergistic effect. No inhibition of the gastrointestinal manifestations induced by α-peltatin was noted during the administration of these hormones, although the febrile reactions appeared to be less than might have been expected with α-peltatin alone. Considerably less euphoria than usually induced by the hormones was noted during the administration of the α-peltatin.

In view of the synergism observed between α-peltatin and antifolic-acid compounds on experimental leukemia, α-peltatin was administered during therapy with a folic-acid antagonist to a boy with acute leukemia of 10 months' duration. This patient had responded twice previously to folic-acid antagonists but appeared to be entering a resistant phase with persistent fever and severe hemorrhagic phenomena. After 8 days of amethopterin therapy, plus antibiotics and numerous transfusions, no evidence of a remission was seen. Two doses of α-peltatin (0.15 and 0.22 mg./kg.) were then given 24 hours apart, while the amethopterin was continued. The patient's fever rose rapidly immediately after receiving α-peltatin. Progressive reduction of hepatosplenomegaly occurred during the next few days. The patient rapidly went into a complete clinical and hematological remission. Whether this event would have occurred with folic-acid-antagonist therapy alone is not known. The combined treatment did not enhance the toxic manifestations expected from each of the agents. A similar complete hematological remission in an adult with acute lymphoid leukemia was also observed after administration of this combination of agents.[8]

D. α-Peltatin-B.—This dextrorotatory isomer of α-peltatin was administered to five patients, each of whom was given a total of 3 to 6 injections; the size of each dose increased progressively in the range of 15 to 250 mg. The cases treated were one each of: multiple myeloma, lymphosarcoma, Hodgkin's disease, chronic myeloid leukemia, and carcinoma of the lung. No physiologic effects whatsoever were observed after the administration of this optical isomer of α-peltatin at dose levels 2 to 10 times greater than those of the naturally occurring isomer. The relative inactivity of the dextro form in animals was thus confirmed in man.

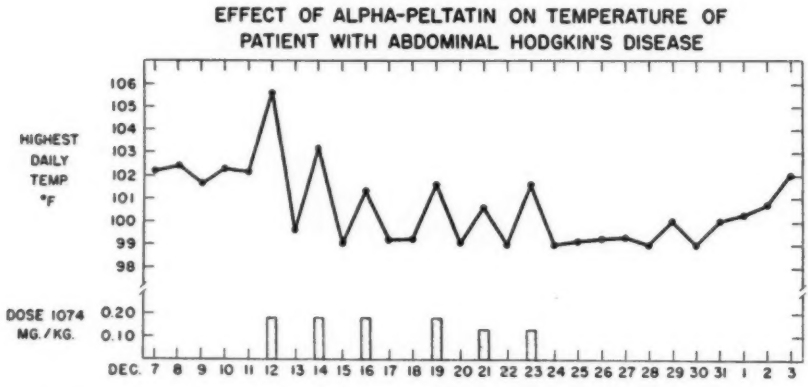
Undesirable Reactions

The systemic responses induced by intravenous α-peltatin consisted of febrile, gastrointestinal, and neurologic manifestations.

Fever (see text-figs. 2 and 3, and table 3) frequently developed within 2 to 4 hours after a dose greater than 0.15 to 0.30 mg. per kg.; the fever subsided 6 to 12 hours after the drug was given. In those instances in which the temperature reached 102° F. or higher, the febrile reaction was usually preceded by a shaking chill.

Fever was observed only twice among patients with carcinoma treated with doses less than 0.25 mg. per kg., whereas doses as low as 0.15 mg.

[8] To be reported later by one of us (E. M. G.).



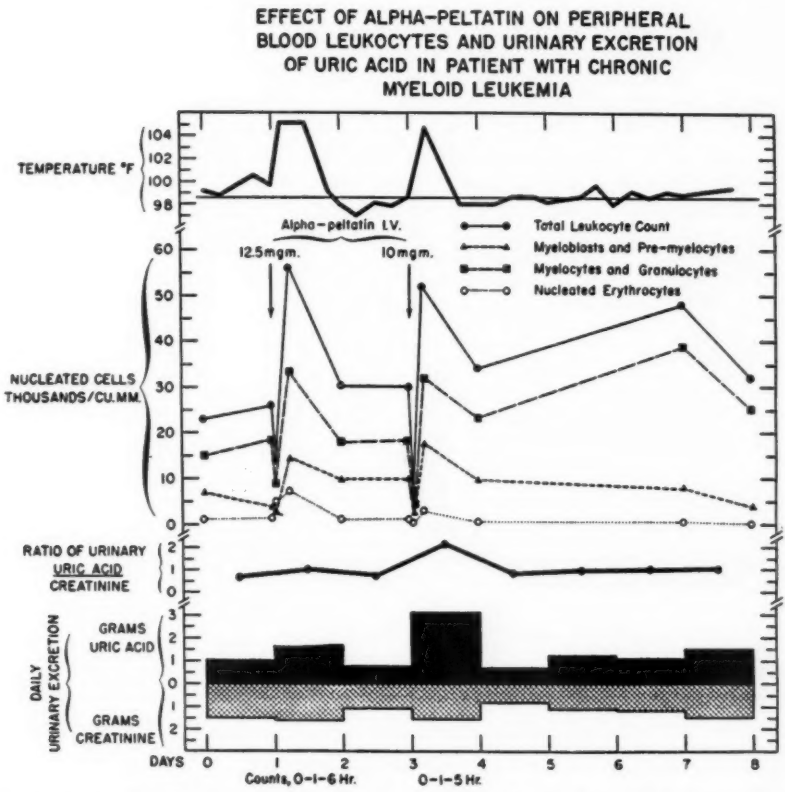
TEXT-FIGURE 2.—Chart showing the highest daily temperature of a patient with abdominal Hodgkin's disease during treatment with α-peltatin. Each dose induced a shaking chill, fever, and gastrointestinal manifestations.

TABLE 3.—*Toxic responses to intravenously administered α-peltatin in 45 patients with advanced neoplasia*

Effect	Number of patients
Asthenia, acute	43
Anorexia, acute	41
Fever, 100° F. or higher	33
Abdominal pain	32
Diarrhea, nonbloody	29
Nausea	16
Pain in lesions	16
Paresthesias	15
Vomiting	8
Peripheral neuropathy (foot drop)	1
Hepatic, renal or cardiac damage	0
Gastrointestinal ulceration	0

per kg. repeatedly produced a febrile response among the noncarcinoma cases. When the dose was reduced below a critical level (approximately 0.1 mg./kg. in patients with lymphomas and 0.2 mg./kg. in patients with carcinoma) febrile reactions were infrequent. There was no evidence of sensitization or progressive tolerance to the drug.

During severe febrile reactions, gastrointestinal symptoms consisting of passage of 1 to 2 bulky semisolid stools, or the development of mild diarrhea, were frequently observed; nausea and vomiting were less frequent. These acute gastrointestinal manifestations usually subsided without treatment within 12 to 24 hours. However, in certain debilitated patients with neoplastic involvement of the gastrointestinal tract, a diarrhea developed which persisted for 2 to 4 days after a single injection. A mild, persistent, nonbloody diarrhea lasting 2 to 4 weeks (3 to 6 stools daily) was frequently observed in patients who had received more than



TEXT-FIGURE 3.—Chart showing the effects of two large doses of α-peltatin in a patient in the terminal phase of chronic myeloid leukemia, previously treated with four courses of X ray and one course of triethylene melamine. Two to three hours following each injection the characteristic febrile and gastrointestinal reaction developed. The patient complained of pain in the region of an enlarged spleen, and generalized pain in the distal portions of the extremities. Moderate shrinkage of spleen (from 16 cm. to 12 cm. below the costal margin) was observed within 24 hours after the first injection, but no effect was noted after the second injection. A course of X-ray treatment for a total dose of 350 r to the spleen was instituted 48 hours after the second dose of α-peltatin. No clinical or hematological response was observed.

three or four doses of α-peltatin which had evoked acute febrile and gastrointestinal symptoms. Opiates, such as paregoric, provided a partial amelioration of the gastrointestinal symptoms.

Consistent changes in the peripheral blood or bone marrow elements were not observed even after doses (0.1 to 0.5 mg./kg.) of α-peltatin that produced diarrhea and fever. Massive normoblastosis (40 to 50 percent of total nucleated count) in the peripheral blood developed, in one of four patients with acute leukemia and one of three patients with chronic myeloid leukemia, within a week after multiple doses of α-peltatin. The

frequency with which spontaneous massive normoblastosis has been reported in the course of such blood dyscrasias rendered difficult the evaluation of the possible role of α-peltatin as a precipitating factor.

The development of hepatic or renal insufficiency which could be attributed to treatment with α-peltatin was not observed despite the fact that many patients were cachetic and in terminal condition.

Transient cardiac arrhythmias appeared in three patients shortly after the administration of α-peltatin. In the first patient, with diffuse metastases (demonstrated at autopsy) in the lung, pericardium, chest wall, and brachial plexus, an auricular flutter supervened within 24 hours after a dose of α-peltatin which induced a febrile reaction, accompanied by severe pain and slight shrinkage in superficial chest-wall lesions. Tissue biopsies taken 6 and 24 hours after injection showed partial tumor necrosis. Necrosis of the neoplastic tissue in or around the pericardium may have provoked the development of this arrhythmia. Two other patients having massive lymphomatous involvement of the supraclavicular, cervical, and superior mediastinal regions, developed a carotid sinus syndrome during the course of treatment with α-peltatin. The carotid sinus syndromes subsided within 10 to 14 days after treatment had been stopped. Because of the local factors involved in the lesions of these three patients, and the spontaneous incidence of arrhythmias following infiltration of cardiac tissue or nerves by tumor tissue, it was not possible to assess the role of α-peltatin in these complications. However, drug treatment appeared to precipitate the onset of the arrhythmia in each instance.

Symptoms suggestive of central nervous system damage were not observed following the administration of α-peltatin. However, paresthesias were noted in many patients (table 3) who received more than two or three large doses. These symptoms were confined to the extreme distal portions of both upper and lower extremities and consisted of hypalgesia and numbness; they subsided 2 to 4 weeks after cessation of treatment. No diminution of deep reflexes, vibration sense, position sense, or deep pressure sense was noted except in one instance. This patient, given the largest single dose in the study (0.46 mg./kg.), developed transient impairment of position, vibration, and cutaneous sensory perception, and diminution of deep reflexes associated with a transient partial unilateral foot drop. These signs of peripheral neuropathy subsided completely 6 to 8 weeks after the last dose had been given.

Metabolic Changes

Urinary excretion of uric acid and creatinine was studied in 17 patients. A moderate uricosuric effect and elevation of blood levels of uric acid were observed in several patients with malignant lymphoma or leukemia after doses of α-peltatin (table 4) that induced a febrile response (text-figs. 1, 3) and diarrhea. This increase in uric acid excretion was accompanied in a few patients by a rise in the creatinine excretion, so that the resulting uric acid/creatinine ratio was not consistently elevated. No change in the urinary excretion or blood levels of uric acid or creatinine

TABLE 4.—*Incidence of febrile reactions induced with a single dose of α-peltatin*

Dose range (*mg./kg.*)	Diagnosis	Patients (*No.*)	Doses (*No.*)	Reactions (*No.*)	Reactions (*percent*)	Patients with reactions (*No.*)	Patients with reactions (*percent*)
0.10–0.25	Carcinoma*	11	18	2	11	1	9
	Lymphoma†	22	104	37	36	14	64
	Total	33	122	39	32	15	45
0.26–0.46	Carcinoma*	15	75	29	39	10	67
	Lymphoma†	14	32	21	66	9	64
	Total	29	107	50	47	19	66

*Including two cases of malignant melanoma.
†Including leukemia, lymphosarcoma, Hodgkin's disease, reticulum-cell sarcoma, and multiple myeloma.

occurred in patients with carcinoma, despite the administration of doses larger than those given to patients with disseminated lymphoma.

No distinct effect of treatment was observed on the levels in serum of polysaccharide, mucoprotein, or β-glucuronidase, on the urinary excretion of 17-ketosteroids and reducing corticoids, or on the eosinophil count in peripheral blood. Because of the low level of the eosinophil counts, and the markedly reduced excretion of the steroids prior to treatment in most of these advanced cases of metastatic neoplasia, the data relating to adrenal function were inconclusive.

Discussion

The undesirable reactions induced by intravenously administered α-peltatin were chills, fever, nausea, vomiting, diarrhea, prostration, paresthesias of the extremities, and pain in the neoplastic lesions. These responses developed 1 to 3 hours after administration of the agent and usually subsided within 4 to 48 hours. The repeated administration of large doses of α-peltatin in debilitated patients was precluded by the development of persistent diarrhea and weakness, especially when the injections were given at intervals of 48 to 72 hours. A transient peripheral neuropathy was induced in one patient in this study. When doses sufficient to induce minimal signs of toxicity were repeatedly employed, there was no evidence of hepatic, renal, cardiac, or central nervous system damage. Thus, it was possible to continue the study of α-peltatin in a relatively large number of patients.

The clinical changes induced by α-peltatin in neoplastic lesions were apparently initiated during the restricted interval of acute pharmacologic response after each of the larger individual doses. Within 24 hours, a rapid reduction in the volume of lymphoid neoplastic tissue was observed in a number of patients with Hodgkin's disease, leukemia, lymphosarcoma, or giant follicular lymphoma. In addition, the sudden development of

tumor slough in several cases of accessible squamous-cell carcinoma was observed. Despite the evidence of acute induced tumor necrosis, progressive tumor growth was often resumed in about 5 days after treatment even with maximum tolerated doses.

The febrile reaction in man may be a consequence of the breakdown of susceptible cells with the release of decomposition products affecting temperature-regulating mechanisms. There was no evidence that the febrile reactions to α-peltatin are antigenic in nature, since the dose at which they were repeatedly reproduced in individual patients remained relatively constant. Moreover, α-peltatin contains no nitrogen, fails to evoke the anaphylactic or the Shwartzman phenomenon (*23*), and shows pharmacologic inactivation after conversion to an optical isomer.

The "therapeutic ratio" and favorable influence of α-peltatin in mice bearing transplantable lymphomas were not reproduced in man. This observation was not unexpected inasmuch as the "therapeutic ratio" for a single dose in rats (*11*) bearing a transplanted carcinoma was only one-fifth that in mice bearing such tumors. Moreover, the maximum doses that larger non-tumor-bearing mammals (rabbits, dogs) could tolerate were considerably lower than those for small rodents. The largest single dose administered to any patient (0.46 mg./kg.) was equivalent to only $\frac{1}{100}$ of the maximum dose (40–50 mg./kg.) tolerated by mice bearing lymphomas.

Further study of α-peltatin with regard to metabolic effects, modification of dosage and method of administration, particularly with regard to potential synergism with other more prolonged-acting antineoplastic agents, is in progress.

Summary

The response to α-peltatin, one of several tumor-necrotizing components isolated from podophyllin, was studied in 45 patients with various metastatic neoplasms. The dosage most frequently employed varied from 0.15 to 0.30 mg. per kg. of body weight, given intravenously at 48- to 72-hour intervals. Reactions developed 1 to 4 hours after treatment with the larger doses and consisted of chills, fever, nausea, vomiting, diarrhea, weakness, pain in neoplastic lesions, and paresthesias of the extremities. Transient peripheral neuropathy with foot drop developed in one patient following administration of the largest single dose used (0.5 mg./kg.). No evidence of renal, hepatic, cardiac, or hematopoietic injury attributable to the drug was observed.

Rapid, temporary regression in splenomegaly and lymphadenopathy, in varying degree, was observed after administration of α-peltatin in a majority of 27 patients with leukemia, Hodgkin's disease, lymphosarcoma, or giant follicular lymphoma. Induced necrosis developed in 3 patients with epithelial neoplasms. These changes occurred only after doses that produced undesirable reactions. The histologic findings in the tumors after treatment were inconclusive. More intensive administration of the drug was precluded by the development of diarrhea and severe asthenia.

The intravenous administration of α-peltatin did not, in this study, yield evidence of significant therapeutic value.

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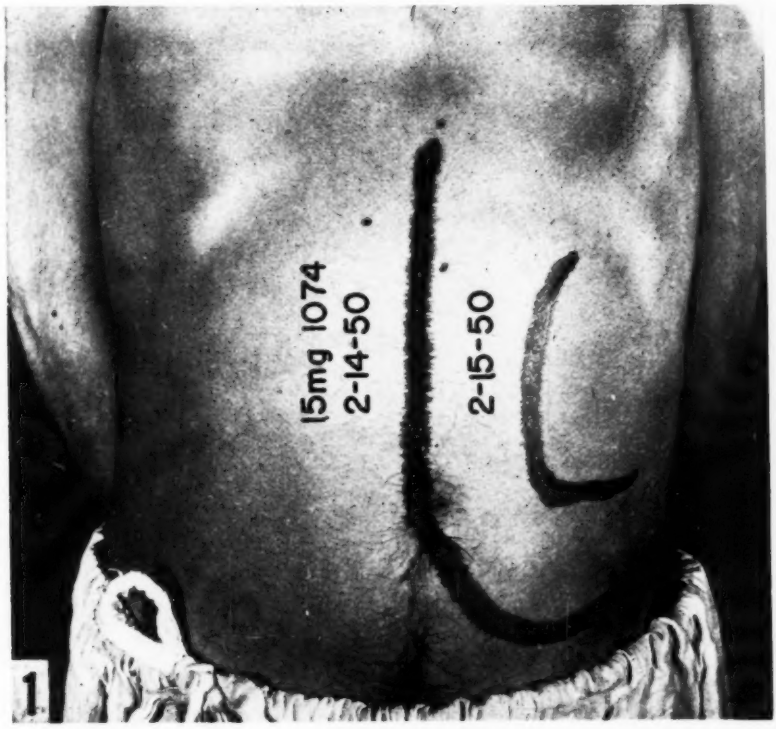
PLATE 89

FIGURE 1.—This photograph shows the change in spleen size 24 hours after the intravenous administration of 0.35 mg. per kg. of α-peltatin in a patient with Hodgkin's disease. Mild febrile and gastrointestinal symptoms occurred 3 to 6 hours after injection of the drug.



Greenspan *et al.* 1273

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PLATE 90

FIGURE 2.—Photograph of a patient with squamous-cell carcinoma of pinna. He had received three previous intravenous injections of α-peltatin at widely spaced (7 to 10 days) intervals. Within 24 hours after the second injection (0.40 mg. per kg.), a diffuse slough had occurred with subsequent regrowth. The figure shows the condition of the lesion just prior to the fourth injection.

FIGURE 3.—Photograph of the same patient shown in figure 2, 24 hours after the fourth injection (0.42 mg. per kg.) of α-peltatin intravenously. A febrile reaction and diarrhea developed several hours after this injection. A massive black slough (biopsy showed diffuse necrosis) is visible throughout the major lesion. The minor lesion, below, was nonfungating and did not show a black slough.

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Mobility and Site of Production of Homograft Resistance Factor [1]

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Previous work from this laboratory has demonstrated the existence of a mobile resistance factor in acquired immunity to tumor homografts [4] (*1*). The essential finding was that when skin grafts were exchanged between immune and nonimmune mice, the growth of a subsequent intragraft tumor implant reflected the immune or nonimmune state of the host mouse rather than the immune or nonimmune state of the skin-graft donor. The two experiments reported in this paper were undertaken in order to determine whether or not similar results would be obtained if the time interval between skin grafting and the implantation of the "challenging" intragraft tumor were shortened, or if the site of prior immunization were incorporated within the skin graft. The first variable concerns the degree of mobility of the previously demonstrated resistance factor, while the second concerns the skin as a possible site of its production.

Methods and Results

The first experiment utilized the techniques previously described (*1*). Strain BALB/cAn male mice 3 to 6 months old were immunized to DBA sarcoma 49 [5] by caudal inoculation. One month later, skin grafts were exchanged between this immunized group and a comparable group of untreated control animals. Within 24 hours after grafting, tumor was inoculated within the skin grafts intracutaneously [*i.e.* superficial to the panniculus carnosus muscle (*1*)]. The average diameter of each tumor was measured at intervals after inoculation. Appropriate simultaneous controls were established as indicated in table 1. This experiment was performed in a manner completely identical to one of those previously

[1] Received for publication January 18, 1954.
[2] With the technical assistance of Earl J. Waters.
[3] National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.
[4] Definitions of terms:
Isograft—a graft between animals of the same genotype.
Homograft—a graft between animals of the same species, but of different genotypes.
Heterograft—a graft between animals of different species.
[5] DBA sarcoma 49, which arose in an animal of the DBA/2 strain, tends to grow progressively subcutaneously in, but to immunize strain BALB/cAn mice. Intracutaneous implants in strain BALB/cAn grow for about 14 days, after which complete regression almost invariably occurs.

reported (*1*) except for the short time interval between skin grafting and the inoculation of the "challenge" tumors. The results, recorded 27 days after the inoculation of the "challenge" tumors, are shown in table 1.

TABLE 1.—*Results of the experiment in which "challenging" tumors were inoculated immediately after skin grafting**

	Immunized recipients			Nonimmunized recipients		
	Number of mice	Number of "takes"	Percent "takes"	Number of mice	Number of "takes"	Percent "takes"
Skin grafts from immunized donors	19	0	0	8	7	87.5
Skin grafts from nonimmunized donors	8	1	12.5	18	15	83.3

*Recorded 27 days after inoculation of "challenge" tumors. A "take" is defined as a grossly visible tumor nodule present at the time of recording.

The second experiment was similar to the first in most respects. However, the immune animals had been immunized 30 days prior to skin grafting by the intracutaneous inoculation of DBA sarcoma 49 [6] by 19-gauge trocar, and the site of immunization was contained within the skin area used for grafting.[7] The "challenging" intracutaneous tumor implantations (which in this case were given with a 19-gauge trocar) were not given until 30 days after the transfer of the skin grafts. In some of the mice an inoculation was made into both the graft and the ventral skin outside of the area of the graft. The results showed no significant difference between these two sites. The data of this experiment, recorded 14 days after the intracutaneous inoculation of the "challenge" tumors, are shown in table 2.

Discussion

The data of both experiments reflected the immune or nonimmune state of the host mice rather than the immune or nonimmune state of the skin-graft donors. Although these experiments were done on a small scale, the data are adequate to rule out the possibility of any major influence of the source of the skin grafts upon the results. More extensive data might well reveal some small influence of the source of the skin grafts, but since such a finding would not alter the conclusions to be drawn from this work, such an extension would be superfluous.

The fact that intragraft immunity was manifest (in the first experiment) within skin grafts from nonimmune donors soon after grafting argues

[6] The DBA sarcoma 49, normally carried in strain BALB/cAn mice, had been transplanted for several transplant generations within (BALB/cAn × DBA/2)F_1 hybrids prior to its use in this experiment.

[7] The skin grafts in this experiment were made very big so that the entire dorsal skin (from high in the neck to the base of the tail and from one extreme flank to the other) was incorporated within the graft. Care was taken, however, not to transplant either axillary or inguinal lymph nodes along with the skin.

TABLE 2.—*Results of the experiment in which the sites of immunization were incorporated within the skin grafts**

	Immunized recipients			Nonimmunized recipients		
	Number of inoculations	Number of "takes"	Percent "takes"	Number of inoculations	Number of "takes"	Percent "takes"
Skin grafts from immunized donors	14	7	50	36	31	86
Skin grafts from nonimmunized donors	32	16	50	18	18	100

*Recorded 14 days after inoculation of "challenge" tumors. The short interval between inoculation and recording, as well as the use of the trocar method of inoculation, accounts for the relatively high number of "takes" in the immunized mice of this experiment, as compared to the first experiment. A "take" is defined as a grossly visible tumor nodule present at the time of recording.

that the resistance factor was very mobile (previous work had shown sufficient mobility to invade a skin graft within 30 days). It seems very unlikely that the invasion of the skin graft by nerve fibers could have proceeded rapidly enough to have played a role in the resistance reaction that occurred. It can, therefore, be concluded that, despite reports to the contrary (*2*, *3*), nerve fibers probably play no direct or important part in the manifestation of an already established homograft immunity.

Since the immune or nonimmune state of the skin-graft donors did not influence the growth of the intragraft "challenge" tumors in the second experiment, it can be assumed that the skin itself is probably not an important site of production of resistance factors in homograft immunity. The results provided no data to indicate that by transplanting directly "immunized" skin a significant site of homologous resistance-factor production was being transplanted. Various authors have presented evidence in the past indicating a local production of antibody during immunization to bacterial antigens (*4*). Perhaps a different result would have occurred in this experiment if the inguinal and axillary lymph nodes had been incorporated within the grafts (*5*).

Summary

Previous work from this laboratory has indicated that when skin grafts are exchanged between an immune and a nonimmune mouse, the growth of a subsequent intragraft homologous tumor implant reflects the immune or nonimmune state of the recipient rather than the immune or nonimmune state of the skin-graft donor (*1*). Thus a mobile resistance factor has been demonstrated. The present experiments utilize similar techniques.

Data are presented which indicate that the resistance factor has sufficient mobility to invade a skin graft within a few days, or even faster. It is, therefore, practically certain that nerve fibers cannot be involved in the manifestation of an already established homograft immunity.

Further data are presented which show that homograft immunity cannot be transferred from an immunized to a normal mouse by the transfer of a skin graft, even if the site of immunization is incorporated within the area of the graft. Therefore, it is improbable that skin, *per se*, is an important site of resistance-factor production.

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Normal and Pathologic Anatomy of the Reticular Tissue in Laboratory Mice, With a Classification and Discussion of Neoplasms[1]

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The need for a comprehensive term to include the whole of the lymphatic and hemopoietic tissue of the body has long existed. Marshall has used the term *reticular tissue,* which he defined as "a tissue composed of fixed cells (primitive reticular cells) supported on a framework of fibrils and including all cells in the body derived from this tissue." His definition included the lymph nodes, thymus, bone marrow and spleen, as the organs that are solely or mainly composed of reticular tissue, and in addition elements of this tissue that exist scattered throughout the body as tissue histiocytes and fibroblasts, the cells of the peripheral blood, and primitive elements that can proliferate to produce any of the cells found in the lymphatic or blood-forming tissue.

Further definition of terms used for some elements of the reticular tissue is required since much confusion exists, particularly regarding the mononuclear, potentially phagocytic cells that can be demonstrated by silver impregnation methods and hence are called "metalophil" cells by Marshall. In the present paper the mature cells of this series are designated as: 1) reticulum cells—solitary fixed cells forming the cellular elements of the reticular framework of the main reticular organs; 2) histiocytes—ameboid cells lying free in the tissues and capable of phagocytosis; and 3) monocytes—cells circulating in the peripheral blood. These three types may be grouped together as "reticulum cells," which is often used as an inclu-

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sive term. In addition there may be distinguished: 4) littoral cells—syncytial fixed cells lining sinuses of the lymph nodes, bone marrow, and spleen; and 5) Kupffer cells—solitary fixed cells forming the reticular tissue of the liver and lining the sinusoids. These different terms are necessary to indicate variations in location and functional activity of what is essentially a single cell form. This line of cells and the other cellular elements of the blood are derived from a primitive reticular cell, which continues as a reserve cell in adult tissues.

Terms used for leukocytes of the peripheral blood are those recommended by the Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood-Forming Organs sponsored by the American Society of Clinical Pathologists, and the American Medical Association.

In referring to the anatomical units consisting of primary and secondary centers in lymphatic organs, the term *lymphatic nodule* is used rather than *follicle*. If reference to the lymph node as "a gland" is obsolete, then "follicle" or "little gland" for the lymphatic unit should be avoided.

Whenever other authors are quoted the original terminology is generally retained, since translation to the newer terminology might be inaccurate.

The present report is concerned with the normal anatomy and the pathologic alterations of the reticular tissue in the mouse. The information has been derived from a review of the literature and from experimental work and observations carried out at the National Cancer Institute.

It should be pointed out that, although for brevity general statements are sometimes made in this review, in dealing with mice—or any other biological medium—generalizations may be inaccurate. Information is reliable only when it refers to specific mice, of known genetic background, age and sex, maintained under laboratory conditions where all extrinsic factors are known and controlled as much as possible.

The subject matter has been arranged as follows:

I. General survey of the literature:
   - A. Normal mice.
   - B. Mice with non-neoplastic disease.
   - C. Mice with neoplastic disease of the reticular system.

II. Examination of the peripheral blood and bone marrow.

III. Blood and blood-forming organs:
   - A. Number and morphology of particular cell types: 1) Erythrocytes, including hemoglobin and hematocrit values; 2) Leukocytes—a) granulocytes, neutrophilic and eosinophilic, b) Lymphocytes, c) Monocytes, d) Plasma cells, including cells with Russell's bodies, and e) Megakaryocytes and platelets.
   - B. Organs of the reticular tissue: 1) Spleen, 2) Bone marrow, 3) Thymus, 4) Lymph nodes, 5) Peyer's patches, and 6) Reticular tissue of the liver.

IV. Non-neoplastic alterations:
   - A. Functional response [extramedullary hematopoiesis is considered under the section on granulocytic leukemia]: 1) Stress reactions and adrenal cortical hormone, and 2) Other endocrine effects.
   - B. Aging and degenerative changes: 1) Aging, 2) Hemosiderosis, 3) Amyloidosis, 4) Cyst formation, and 5) "Mesenteric disease."
   - C. Hyperplasia of particular cell types: 1) Reticulum cells, 2) Plasma cells, including cells with Russell's bodies, and 3) Mast cells.

D. Reactions to injury: 1) Unidentified irritants with manifestations of acute, chronic, or granulomatous inflammation; 2) Living organisms: a) Known bacterial and viral agents, b) Unidentified agents; 3) Mitotic poisons; 4) Irradiation; and 5) Growth of tumors.

V. Neoplastic and related conditions:

A. Terminology and classification. Lesions derived from: 1) Stem cell, 2) Lymphocyte, 3) Granulocyte, 4) Reticulum cell, 5) Plasma cell, 6) Mast cell, and 7) An unclassified and miscellaneous group.

VI. Lesions found in mice of various strains and types of hybrids at the National Cancer Institute.

VII. Significance of neoplastic lesions of the reticular system in mice in relation to disease in man.

I. General Survey of the Literature

Only the most important and comprehensive papers relating to the general subject of the reticular tissue of the mouse are cited in this section. Papers dealing with particular cell types, organs, or physiologic or pathologic processes are reviewed in later sections. When a recent, comprehensive review of a particular subject is available, this alone may be cited. Consequently, reference to important papers may be omitted if they are cited and discussed elsewhere. On the other hand, minor reports are sometimes discussed because they have been generally overlooked. It was often difficult to set a limit to the discussion, especially now that the reticular tissue of the mouse is a favorite biological medium for testing various endocrine substances and irradiation effects, and papers on this subject are rapidly accumulating.

A. Normal mice.—Gardner stated, in a review of the literature from 1936 to 1946 on the blood picture of different laboratory animals, that "not much information is available on the blood of normal mice, since these animals have been used very little in hematologic work." She cited important reports during this period by Fekete, Dougherty and White (1944), Gowen and Calhoun, Goulden and Warren, and Francis and Strong. Most of the work was done on inbred strains. Publications prior to 1936 were reviewed in a report by Emile-Weil and Bousser, and they also gave figures on the number of cells in the peripheral blood, derived from their own studies. Another early review by Scarborough on the hematology of laboratory animals included the mouse, and references were given to publications by Klieneberger and Carl, Simonds (1925*a*), Petri (1933, 1934), and de Kock. The papers by Petri were comprehensive, and included many of his own observations and descriptions of his techniques. Haam also published data on this subject. This work, however, preceded the use of inbred strains and is not of much immediate value today. Grüneberg (1952) also gave a brief review of the blood of the normal mouse, and a more comprehensive discussion of the several hereditary anemias appearing in certain inbred strains. Russell *et al.* (1951) compared the normal blood picture of young adults from 18 inbred strains, and Endicott and Gump described the hemograms and myelograms of mice from two inbred strains. These papers emphasized the differences among the strains, and also indicated the range that was found in the hemograms of

mice of the same strain under presumably identical conditions. While figures as to the number of cells in the blood of "normal" mice may be available, controls of the same strain, age, and sex, maintained under the same conditions, are advisable in any hematologic experiment.

As with the blood, very little has been written on the normal anatomy of the blood-forming organs. Fekete gave brief descriptions and important references on histology. Changes in normal mice dependent upon hormonal stimulation have been described by Andreasen (1948), Dmochowski and Horning, Dougherty and White (1944, 1945), and Kaplan, Nogareda and Brown. These are discussed more fully later.

Because so few reports on the hematology of the mouse are available, several studies on the rat are cited. Descriptions have been given by Andreasen (1943) of lymphatic tissues in the rat and the changes accompanying food restriction, by Andrew of age changes in the spleen, and by Andrew and Andrew of age changes in the cervical lymph nodes. Kindred (1938) made a quantitative study of the lymphoid organs. Gillman and coworkers described the cytology of the lymph nodes in control rats and in rats treated with trypan blue, and discussed the origin of various cell types (1952). The variability in the structure of the lymph nodes in healthy rats was emphasized. Reinhardt recorded the weights of lymph nodes, thymus, and spleen, and the output of thoracic-duct lymphocytes in normal rats of the Long-Evans strain at different age periods.

B. Mice with non-neoplastic disease.—Descriptions of pathologic alterations in the blood-forming organs are found in papers by Parsons on the amyloidosis developing after pentose nucleotide injections (1945), and the plasma-cell increase after various experimental procedures (1943); by Dingle on the changes produced by infectious organisms; and by Barnes and Sisman on leukemoid reactions and accompanying changes in the blood-forming organs. Reactions to a variety of experimental procedures are found in widely scattered reports, and no attempt is made to review them here.

C. Mice with neoplastic disease of the reticular system.—Reviews by Engelbreth-Holm, Andersen, Heim and Schwartz, and Kaalund-Jørgensen (1936) may be consulted as guides to the earlier reports. Cloudman (1941) also cited important references, and Richter and MacDowell (1935) gave a comprehensive review of their studies in one inbred strain. A review by Furth (1946) on experimental leukemia, and a more recent paper by the same author (1951) gave references to the most important reports on the morphology and biologic behavior of leukemia in the mouse. A review article by Kirschbaum discussed recent biologic studies on leukemia in rodents. The morphology of the neoplasms has been described in most detail in papers by Haaland (1905, 1911), Tyzzer, Murray, Jobling, Simonds (1925*b*), Andersen, Kaalund-Jørgensen (1936), and in many papers by Furth, MacDowell, and their associates.

References to particular forms of neoplasia are discussed later along with descriptions of the individual types.

II. Examination of the Peripheral Blood and Bone Marrow

Before hematologic studies in the mouse are undertaken, technical methods should be investigated. In general the results are dependable, but there are some sources of error. In any publication reporting hematologic findings in the mouse it is advisable to describe the methods employed, since these are not sufficiently standardized for general reference.

Examination of the Peripheral Blood

With a few modifications necessary because of the limited amounts of blood that can be drawn, the same methods are used as in hematologic studies in man. A single blood determination in the mouse requires a relatively large quantity of blood and, if possible, frequent withdrawals from the same mouse should be avoided. Arvy (1944*a*) advised that blood counts on the same animal should not be made oftener than every 15 days.

The blood is commonly withdrawn from the tip of the tail, and a special holder that keeps the animal immobile and allows access to the tail is helpful. The lateral or ventral tail vein is cut, and the first drops wiped away. The blood should flow freely, and the tail should not be squeezed since this may alter the number and proportion of the leukocytes. Russell *et al.* (1951) gave nembutal anesthesia before hematologic procedures, since lower counts and less variability were found in animals so treated, and they heated the tail in hot water before cutting the tip. Subsequent work by Budds *et al.* showed that in most strains nembutal lowers the leukocyte count, but this is not universally true. Strain C57BL/6, in contrast to all others, gave higher granulocyte counts, and strain A showed extreme lowering with nembutal. This observation is significant in the interpretation of any experiments involving leukocyte counts in mice. The Randolph method for chamber enumeration of granulocytes and agranulocytes in phloxine–methylene-blue–propylene-glycol diluent was tested and found applicable to mouse blood.

Ershoff and Gaines, in experiments with rats, reported that heating the entire body had some advantages over heating only the tail.

Methods for withdrawing large quantities of blood from the mouse—pure venous or pure arterial blood from the tail, and sterile blood by heart puncture—are described by Ambrus *et al.*

Hemocytometers such as are used in human hematology are satisfactory. Erythrocyte counts in this laboratory have been made with special pipettes requiring a smaller volume of blood than the pipettes used for humans, but satisfactory results may be obtained with the latter. The quantity withdrawn should be reduced for very young or extremely anemic animals.

Reticulocyte counts may be made after staining the blood smear with cresyl violet, followed by counterstaining; or the fluid blood may be mixed with the stain, covered with a cover glass, and examined and

counted while wet. The method for staining and counting reticulocytes described by Brecher is satisfactory for mouse blood if care is taken to avoid excessively thin smears in which a good number of reticulocytes are traumatically hemolyzed, giving a confusing picture of "reticulum out of the cell."

The leukocytes may be counted with the pipettes ordinarily used for human blood, using the standard volume, or a smaller volume of blood than indicated for man may be drawn; or special pipettes for small animals requiring a small volume of blood may be obtained. Methods for counting eosinophils to determine the response to adrenal cortical hormones were described by Speirs and Meyer, and Halberg *et al.* used similar methods to determine the eosinophil levels under standard conditions.

Platelet counts are usually done by a direct method using Rees-Ecker diluting fluid. Brecher *et al.* (1953) described a direct method using the phase microscope, and by this method the platelet counts appeared to be as reproducible in the mouse as in man. Marked variation was noted from mouse to mouse, even of the same strain.

Hemoglobin determinations have been made in this laboratory with the Coleman Junior spectrophotometer by the acid hematin method. Turner's cyanomethemoglobin micromethod was used by Russell *et al.* (1951). Other laboratories have used the Evelyn photoelectric colorimeter, and an alkaline hematin method. Since it is usually the relative variations in the hemoglobin readings that are important, the same instruments and methods should be used throughout an experiment. Adequate standardization of the instruments should be made before comparing results from different laboratories.

Hematocrit determinations have been made in this laboratory by an ultramicromethod described by Natelson. A microhematocrit apparatus, described by Strumia and Principato, is now being tried, and the results have so far been reliable when compared with other methods. Russell *et al.* (1951) used the Van Allen hematocrit tube.

A method for the determination of blood volume has been described by Wish *et al.*, and by Oakley and Warrack. Plasma and blood volumes of mouse organs were determined by Kaliss and Pressman using radioactive iodoproteins. Grüneberg (1941) determined the blood volume from birth to the weaning period.

Antigenic differences in the blood among different strains of mice have been studied by Gorer (1947) and by Davidsohn and Stern. Their reports gave details of the methods used, and discussions of previous work. The *p*H values in inbred strains differing in their resistance to mouse typhoid have been determined by Weir (1949). This paper gave references to important preceding work comparing various factors in strains resistant to or susceptible to mouse typhoid. Weir (1953) also reported that a line of mice having a high blood *p*H gave birth to 52.8 percent males, while a low *p*H line had 42.8 percent males.

Platt and Zeller determined the bleeding time by the Duke method,

and reported average values of 2 minutes, 15 seconds. Coagulation time which they determined by the capillary-tube method was 4 minutes, 20 seconds. Kreisler reported the blood-clotting time in C3H/Jax mice as 49 seconds. This was greatly increased following the injection of heparin.

Blood smears may be made by the same methods used in human hematology. Either Wright's or Giemsa's stain, or a combination of the two, have been used. Dr. Clarence Velat of the National Cancer Institute recommends the following method for staining films of mouse blood (figs. 1–6):

Drying: Allow films to dry overnight, or keep in an incubator at 37° C. for 3 to 4 hours.

Timing: Make a trial staining of a sample film, using 1 cc. of "stock" stain for 1½ min. and 1 cc. buffer or distilled water for 3 min. Depending upon intensity of staining obtained, modify the timing for use on similar films. A Coplin dish is advised if handling large numbers of films.

Materials: Wilson's powdered blood stain (a Wright-type stain obtained from the Coleman and Bell Co.), May-Grünwald (sold by National Aniline Division of Allied Chemical and Dye Corp.), and absolute methyl alcohol (C. P. Merck).

Preparation: Add 0.3 gm. of Wilson's stain, a little at a time, dissolving thoroughly after each addition, to 100 cc. methyl alcohol kept at 50° C. in a water bath. This makes a 0.3-percent solution.

Prepare similar solution of May-Grünwald stain. This will keep indefinitely.

Add 1 part May-Grünwald to 3 parts Wilson solution, to make "stock" stain. Store in a dark bottle. It is immediately ready for use, and may be kept for a period of several months.

Procedure: Pipette 1 cc. of stock stain on blood film. Let remain for time determined by trial staining.

Add 1 cc. of Haden's phosphate buffer, *p*H 6.4, or distilled water. Mix by blowing gently on slide. Leave for time determined by trial staining.

Wash slide in distilled water. Examine while wet, and if too light, restain from beginning. If too dark, submerge in a beaker of distilled water, and examine periodically until proper intensity is reached.

Cells to be noted in doing a differential count are described later.

De Kock described methods and reported observations on supravital staining of blood in mice. He also described the locomotion of mouse leukocytes in tissue culture.

The phase microscope permits observations on the freshly drawn blood of mice, and may give information as to the relative number of reticulocytes, the presence of Heinz bodies, the types of leukocytes, and the morphology of abnormal cells. Phase-microscope observations should always be followed by the usual hematologic procedures. As yet the phase microscope has not revealed anything new regarding blood cells of the mouse, but it confirms data obtained by other methods.

Examination of the Bone Marrow

Bone-marrow impressions or smears may be made from the femoral marrow, or marrow from other sites. Differential cell counts of the marrow are often of doubtful value because of uncertainty in identifying primitive cell types and the unequal distribution of cell types in the smears. Brecher

and coworkers (1948) have described an exacting technique by which a quantitative estimation of the different cell types was obtained. This technique required a differential count (using a method described by Endicott), which was correlated with an estimation of cellularity determined from photomicrographs of the bone marrow from the same animal. Although this method is tedious and is not recommended for general use, it gives very reliable data. Russell *et al.* (1953) used it to study marrow from the sternum and femur of normal and anemic mice, and by this means they could demonstrate maturation arrest.

Differential counts of marrow cells were reported by Price-Jones, Simonds (1925*a*), Jaffé (quoted by Haam), but there is some doubt as to the reliability of the results when compared with more recent observations. Petri (1934) also made differential counts on marrow, but as he stated, the counting was tedious and because of its rather limited usefulness was of more theoretical than practical importance. He used a direct-impression method without dilution. Criteria for distinguishing different cell types were given. It was concluded that lymphocytes in the marrow were derived from the blood, and that plasma cells were normally found in the marrow. Petri advised, however, that in most types of work, microscopic sections were sufficiently reliable for making estimates of the relative numbers of cell types.

To be valid, conclusions drawn from hematologic studies in mice require the use of inbred strains in which a uniform blood picture is expected, and an adequate number of controls should be handled in the same way as the experimental mice, permitting close comparison between the two groups. The number of circulating leukocytes is so labile, even in mice presumed to be normal, that considerable variation from the range found in the controls is required before the difference becomes significant. Variations in the external temperature, excitement, and exercise may all cause wide fluctuations in the leukocyte count. Differences due to variations in technique are also considerable, even when the same person does all the procedures.

The same animal should not be bled at frequent intervals, and if any complicating disease is present the animal should be discarded. It is also important that the findings in the peripheral blood should be supplemented by autopsy studies including sections of all the blood-forming organs. The peripheral blood is not an isolated and independent tissue but a part of a complicated system.

The mouse offers many advantages for hematologic studies because large numbers of animals are easily maintained under experimental conditions. Autopsies are not difficult or time-consuming and a complete survey of all organs can be made.

III. Blood and Blood-Forming Organs

This section reviews the most important papers, and reports observations made in this laboratory.

A. Number and Morphology of Particular Cell Types

The review by Gardner forms the basis for the figures given below for each cell type. Gardner published exact figures from many different sources, but only approximate averages derived from her report are given here. Another source of information is the paper by Russell *et al.* (1951), who studied the formed elements of the blood of young adult mice of 18 different inbred strains.

1) Erythrocytes, Including Hemoglobin and Hematocrit Values

The number of circulating erythrocytes in Gardner's review ranged from about 7 million per cu. mm. in the Bagg albino strain of mice to over 11 million in strain DBA. De Kock reported 10 million for Rockefeller Institute and Swiss mice, and noted that in contrast to the leukocytes, the number of erythrocytes was generally uniform. Kamenoff cited preceding reports and gave his own results of erythrocyte counts in four inbred strains of mice: Bagg Albino, d.br., C58, and Sto-Li. There was practically no difference between the means in the four strains, but the mean red-cell count of the males was slightly higher than in the females, being 10,019,400 ± 68,900 for the males, and 9,225,000 ± 46,700 for the females. A wide range was noted in individuals in the different strains. Klieneberger and Carl also reported higher erythrocyte counts in males than in females. Russell *et al.* (1951) found a range from 8.79 million per cu. mm. in strain C3H to 10.54 million in strain BALB/c.

The erythrocyte life span in the mouse was calculated at about 25 days by Harrison *et al.*, using radioactive iron as a tracer. By the same method the life span in the dog was determined as 110 days, and in the rabbit and rat as 50 days.

The hemoglobin values reported have also varied, ranging from 12 to 17 gm. per 100 cc. in Gardner's review, from 19 to 20 gm. in de Kock's report, and in inbred strains reported by Russell *et al.* (1951), from 12.1 gm. in strain R-III to 15 gm. in strain BALB/c. Hemoglobin values per cc. of cells given by Russell *et al.* (1951) ranged from 0.29 in strains DBA, C57BL, and C57BR to 0.31 in strains BALB/c, CBA, C3H, I, and R-III. Francis and Strong reported that hemoglobin values were reduced with advancing age.

Hematocrit values given by Russell *et al.* (1951) ranged from 39.5 percent in strain C3H to 50.6 in C57L, and the mean cell volumes ranged from 41.6 cubic microns in strain CBA to 48.9 in strain AKR, except for a very high value of 51.5 in strain C57L. It was noted that in the great majority of strains variations in mean hematocrit values could be explained by variations in cell number. One strain only, C57L, showed a mean cell volume significantly higher than that for all other strains. Values of hemoglobin per cell were remarkably uniform.

Variations in erythrocyte number and hemoglobin content dependent upon hormone effects are discussed later.

Size and morphology.—The erythrocyte is smaller in the mouse than in man, measuring only 5.7 mm. in diameter as given by Kerti and Stengel. Each cell has a smaller content of hemoglobin than is found in man and the centers appear pale (fig. 2). Basophilic erythrocytes are relatively frequent, usually numbering 10 percent or more. The reticulocytes in the mouse are noticeably larger than the mature erythrocytes, and number from 1 to 6 percent with an average of 4 percent, according to Krumbhaar (1922). Russell *et al.* (1951) reported values ranging from 1.5 to 3.5 in 18 inbred strains of mice. Seyfarth and Jurgens recorded 60 to 70 percent reticulocytes at birth, with a quick decline to 5 to 6 percent a few days later. Nucleated red cells are rarely found in the peripheral blood of adult mice. The blood of newborn mice shows considerable anisocytosis and polychromatophilia, with many very large basophilic erythrocytes (fig. 1).

Identification of early stages in erythrocyte development is difficult in the mouse, since a sharp separation in the morphology of primitive cells is not seen in this species.

Several pathologic processes that affect the erythrocytes have been described. Two hereditary forms of anemia have been intensively studied, mainly from the genetic aspect. Grüneberg (1952) has reviewed preceding work and reported observations of his own (1942, *a* and *b*). One type appeared in a strain in which flexed tail and a belly-spot were also found and were presumably controlled by the same recessive gene. This anemia developed *in utero*, and showed a more severe reduction in the hemoglobin than in the red-cell count. Recovery proceeded rapidly after birth, and the cell count might be nearly normal by the end of the first week. Normal hemoglobin levels were attained less rapidly. A remarkable feature of this anemia was the presence of siderocytes, which are erythrocytes containing free iron in a granular form that can be stained by the prussian-blue reaction or by other methods for demonstrating iron. Even after apparent complete recovery from the anemia, a small percentage of siderocytes could be found in the flexed-tail mice. Similar cells have been found in small numbers in normal mice, in human embryos, and in human beings with anemia, described by Case.

The second hereditary anemia, which also affects the embryo before birth, has been recently studied by Russell and coworkers (1953). It is macrocytic in type, and is completely refractory to liver, liver extract, and folic acid.

Russell *et al.* (1953), using a method of estimating the cellularity of mouse bone marrow described by Brecher *et al.* (1948), concluded that the anemia was normocytic rather than megaloblastic, and that the *W*-series genes blocked some stage of normoblastic erythropoiesis. Russell investigated the connection between the action of the *W*-gene leading to blood changes and that leading to pigment intensity. Severe anemics were black-eyed whites, and germ cells were lacking in both sexes. Russell and Fondal studied four alternative degrees of the anemia dependent upon different manifestations of spotting genes. Valuable methods for

the investigation of blood in the fetus and very young mice were given in this report.

The erythrocytes of the mouse develop Heinz bodies readily after exposure to a number of toxic agents. Webster has reviewed previous reports, and described his own observations.

2) *Leukocytes*

The granulocytes, the lymphocytes, and the monocytes are the types normally found in the peripheral blood. The average total number from inbred strains ranged from 8,000 to 22,000 per cu. mm. with an average of around 14,000 (Gardner). A considerable difference was found even in presumably normal animals in the same inbred strain, and Gardner's figures showed that the number of leukocytes in one mouse in a group might be more than twice the number of leukocytes found in another. Russell *et al.* (1951) found mean values ranging from 5.07 thousand per cu. mm. in strain C3H to 11.62 thousand in strain I. A considerable difference has been observed between blood drawn from the tail and from the heart, the blood from the tail having a much higher number as reported by Fekete. De Kock counted leukocytes in blood drawn from the tail and from the aorta. He found the total count from the tail was 1.4 to 5 times as high as that from the aorta, whereas the differential counts coincided closely. We have observed that blood drawn from the right ventricle has a consistently higher number of leukocytes than blood drawn from the left ventricle, and blood from the tail contains a much higher number than heart blood.

Eschoff and Gaines reported that the peripheral leukocyte count in the rat was significantly lower and comparable to that of heart blood if the entire body was heated and not the tail alone. References to reports on the difference between heart and peripheral blood counts in the rat were cited.

An increase in the number of leukocytes with age is recorded by Gowen and Calhoun. De Kock carried out a series of counts and concluded that even when such factors as diurnal rhythm, mechanical influences, illness, age, sex, strain, dietary regimens, and environment were controlled, the variations in the number of leukocytes might be great, with extremes of 4,000 to 39,000, although most total leukocyte counts fell into a range from 11,000 to 18,000. Autopsies were performed by de Kock on animals with extremely low or extremely high total counts, and as a rule no disease could be found to explain the variation.

It was reported by Gowen and Calhoun that, in general, strains of mice resistant to *Salmonella typhimurium* possessed relatively high leukocyte counts. Weir *et al.* produced a high-leukocyte line (counts above 15,000) and a low-leukocyte line (counts below 7,000), and found that a high leukocyte count did not, by itself, make the host more resistant to mouse typhoid. This supported their view that resistance was dependent on complex interactions of characters which taken independently did not confer resistance.

Arvy (1944*a*) observed that the number of leukocytes in ovariectomized mice and male mice (1944*b*) was relatively constant, whereas that of intact female mice fluctuated. Variations which she found due to the estrus cycle and to hormone injections are discussed later. Her observations suggest that the male or a castrate female furnishes a better standard animal for testing the effect of various substances on the peripheral leukocyte count than does the intact female. Dr. Elizabeth Russell, on the contrary, has generally noted more variation in male mice (personal communication).

a) Granulocytes,[4] *neutrophilic*

Number.—This form comprises about twenty percent of all leukocytes, but may show pronounced increases in pathologic conditions. Russell *et al.* (1951) noted that there was great strain variability in the absolute number of lymphocytes, and that the mean total leukocyte count for a strain was negatively correlated with the proportion of granulocytes. Thus, the smaller proportion of granulocytes, the higher the total count. The percentage of granulocytes was slightly higher in the male, an average of 19.4 percent in comparison with 16.5 percent in the female.

In a later paper Budds, Russell, and Abrams proved that there was a significant strain difference in the number of both granulocytes and agranulocytes (largely lymphocytes), and that the two cell types varied independently of each other. Since the lymphocytes develop in lymphoid tissue, and the granulocytes in bone marrow, this was not necessarily surprising. Correlation of granulocytes with numbers of erythrocytes, which also develop in the bone marrow, was not found in all strains. This indicated that different genes affected different cell types. No relation was found between the number of lymphocytes at 2 to 3 months, and future potentiality for lymphatic leukemia.

Morphology.—In the peripheral blood, the nucleus of the neutrophilic granulocyte may be multilobed with nodular masses of chromatin separated by narrow strands (fig. 2). It is easily identified with the phase microscope in freshly drawn blood, where its characteristic locomotion can be observed. In tissue sections and smears the nucleus is often "ring" or "doughnut" shaped. The granules are fine and do not stain as readily as those in human granulocytes.

The morphology and development of the granulocyte in the bone marrow have been described by several investigators. Endicott and Gump considered that important reasons for the difficulty in identification of cell types in the bone marrow were: 1) the scarcity or complete absence of cytoplasmic neutrophil granules in the precursors of the granulocyte, and 2) the maturation of the granulocyte by the formation of ring nuclei, in contrast to the more distinctive metamyelocyte or stab forms of human marrow. These authors advised the use of peroxidase preparations routinely for the identification of the granulocyte series in the bone marrow.

[4] The term "heterophil" applied to the granulocyte in the peripheral blood of laboratory animals is no longer justified, since the identity of this cell with the granulocyte of human blood can no longer be questioned, and the different terminology causes confusion.

In a later paper with Brecher as senior author it was stated that the nucleus and cytoplasm of the granulocyte might not mature concurrently, which added to the difficulty in identifying this cell. The authors advised that attempts should be made to identify only three classes of cells: 1) young forms consisting of relatively large cells with basophilic cytoplasm, with or without azurophilic granules, and having round or early ring-form nuclei containing finely divided chromatin; 2) old forms consisting of cells with pale basophilic or neutrophilic cytoplasm, and with ring-form nuclei showing early indentation or fully developed segmentation and with coarsely distributed chromatin; and 3) intermediate forms, including all other cells of the granulocytic series. In the last group the granules of the cytoplasm were partially mature and the stage of development of the nuclear chromatin was not always consistent with the development of the cytoplasm.

Petri (1934) when studying the bone marrow reviewed opinions as to the formation of the "ring" nucleus. It was generally agreed that it developed from a solid or oval nucleus by a thinning out of the central part, although some observers thought a rod-shaped nucleus was first formed, and that it later curved so that the two ends were joined.

Granulocytes, eosinophilic

Number.—Halberg *et al.* determined the absolute eosinophil levels in the tail blood of mature male mice of different strains. A characteristic number was found for each strain investigated, and under rigidly controlled, standard conditions these results were reproducible. The highest mean value for any strain was 1,022 and the lowest was 129, which indicated the wide range of difference between the strains examined. Differences of 2- to 10-fold were found in mice of the same stock. A regular diurnal physiological variation was found in 5 stocks of mice by Halberg and Visscher. On the average, the midnight count was less than one-third of the morning level. Differential eosinophil counts reported in Gardner's review have ranged from 0.0 percent to 15 percent, with an average of 2.58 percent. Reduction in the number of eosinophils in the peripheral blood of the mouse is used as a test of stress reactions and hormonal effects to be discussed later.

The variability in number of eosinophils in mice requires that only inbred mice under controlled conditions be used as test objects.

Morphology.—The eosinophil has a ring-shaped nucleus and the cytoplasm contains large, uniform granules which stain less distinctly than granules in human eosinophils (fig. 2). In the peripheral blood the nucleus does not develop the degree of segmentation often seen in neutrophilic granulocytes. It is motile when observed in freshly drawn blood under the phase microscope; the granules vibrate during motion of the cell, and appear to be discoid in shape. A clear area free of granules is often seen in the cytoplasm within the ring-shaped nucleus. The eosinophil is readily identified in tissue sections after Giemsa staining but may be difficult to recognize with hematoxylin and eosin.

Blood basophils are not normally found in the mouse. An occasional cell with metachromatic granules may be found in blood smears, but these are probably tissue mast cells which were expressed from the tissues when the smear was made.

b) Lymphocytes

Number.—This is the most frequent type of leukocyte in the peripheral blood, forming about sixty to eighty percent of all white cells. Russell *et al.* (1951) found that the mean percentage value was 80.6 in male mice from 18 inbred strains and 83.5 in females. A great strain variability was noted in the absolute number of lymphocytes. Russell and her coworkers were testing healthy young mice in which granulocyte percentages were relatively low and constant. The number varied under abnormal conditions, and after certain types of injury and hormonal stimulation to be discussed later.

Morphology.—The lymphocyte is slightly larger than the erythrocyte and typically has a round nucleus with abundant chromatin, and a very narrow rim of basophilic cytoplasm (figs. 3, 4). Larger forms are found in which the nucleus may be slightly indented, and the cytoplasm more abundant. This larger form of the cell may be difficult to distinguish from the monocyte (figs. 3, 4), a fact pointed out by Furth (1939), de Bruyn *et al.*, and Gorer (1946, *a* and *b*). Gorer (1946*a*) stated that while differentiation between these cell types is generally easy in man, it is difficult in the mouse.

c) Monocytes

Number.—Considerable variation is found in figures giving the percentage of monocytes in total leukocyte counts. The recorded percentages range from 0.7 to 14 with an average of about 6, reported by Gardner. Russell *et al.* (1951) did not classify monocytes in their report.

Morphology.—Furth (1939) and Gorer (1946*b*) have given detailed descriptions of this cell. It is the largest of the leukocytes, measuring about 12 μ according to Gorer, and it has a round, oval, or bean-shaped nucleus and a nongranular, slightly basophilic cytoplasm (fig. 4). Vacuoles may appear in the cytoplasm. Gorer discussed the variations this cell may undergo, and the similarity that the smaller forms may have to the lymphocyte.

d) Plasma cells

The plasma cell is not ordinarily seen in blood smears, but may be numerous in tissue sections, especially in lymphatic tissue of old mice.

Morphology.—The plasma cell is readily identified by its oval shape, its eccentric nucleus which may show a characteristic wheel-spoke arrangement of the chromatin, a light-staining area in the cytoplasm near the center of the cell (the cytocentrum), and a characteristic violet color of the cytoplasm after Giemsa staining. The plasma cell is frequently modified in the mouse and contains Russell's bodies. In this form it is characterized by a small, heavily basophilic nucleus, and by globular bodies within

the cytoplasm, which take an intense pink stain after periodic acid-Schiff treatment.

e) Megakaryocytes

Distribution and number.—Megakaryocytes do not appear in the peripheral blood. They are prominent cells in the bone marrow and spleen of healthy mice, and may appear at any other site in which extramedullary hematopoiesis takes place. Degenerating forms may be observed in the lung. Megakaryocytes may be increased in the spleen when there is an increased hematopoietic activity, especially of the granulocytic type. Parsons and Warren described changes in the number of "giant cells of the spleen" (megakaryocytes) in mice subjected to a number of procedures. The "giant cells" were greatly increased in mice with subcutaneous grafted sarcomas, and decreased when the grafts were intraperitoneal. They were also decreased in mice having primary induced sarcomas, or receiving injections of a carcinogenic compound, or after X radiation. There was a significant degree of correlation between the blood leukocyte counts and splenic "giant cells" in mice with subcutaneous grafted sarcomas or primary induced sarcomas. Platt and Zeller described an increase in megakaryocytes in the spleen and the lung of mice of three strains following the injection of an extract from human spleens that had been removed for essential thrombocytopenic purpura. This work has not been confirmed, and the existence of a specific stimulating factor would be difficult to determine since an increase in megakaryocytes develops under many conditions, and the absolute number cannot be accurately determined. The megakaryocyte is normally present in the liver at birth, and information is available regarding the age of disappearance, which usually takes place in the first few weeks of life. Distinct strain differences were noted by Enzmann and by Fortuyn.

Platelets

Number.—From 246,000 to 987,000 platelets per cu. mm. of blood are reported by different observers cited by Gardner. Platt and Zeller reported average platelet counts of 389,000 in normal mice of strains A, Swiss, and C57. The platelets decreased after the injection of extracts of spleen from patients with essential thrombocytopenic purpura to an average of 160,000, with a gradual return to 410,000. Brecher (personal communication) found platelet counts generally well over 1,000,000 in a group of mice from the National Institutes of Health. The phase microscope was used in making the counts (Brecher *et al.*, 1953), which were found to be as reproducible in the mouse as in man. Many different stimuli affect the platelet number.

Morphology.—The platelet bodies are similar to platelets in man (fig. 2). Giant platelets are sometimes found. In freshly drawn blood the platelets appear under the phase microscope as plump oval bodies with numerous fingerlike adhesive projections. Within a few minutes these free bodies

are converted into the platelike structures commonly seen in blood smears.

B. Organs of the Reticular Tissue

The spleen, bone marrow, thymus, lymph nodes, Peyer's patches, and the reticular tissue of the liver comprise the important reticular tissues of the mouse. Histologic sections of these organs stained with hematoxylin and eosin, Giemsa, and toluidine blue, and treated by the periodic acid–Schiff procedure and by the prussian-blue method were collected from mice of strains DBA, BALB/c, A, C57L, and C58 in this laboratory. Five male and five female mice of each strain were examined at 6 weeks of age and at 12 months. The information given below was acquired principally from examination of this material. Observations made from the examination of other control and experimental mice used at the National Cancer Institute have also been utilized, as well as the published reports cited.

1) Spleen

Location, form, and size.—The spleen of the mouse lies diagonally in the upper left quadrant of the abdomen (fig. 7). It is elongated, ovoid, and slightly curved. The surface adjacent to the abdominal wall is rounded, and the splenic vessels enter the opposite, slightly concave surface, which is in such close contact with pancreatic tissue that, microscopically, splenic tissue at the hilus may be found extending into pancreatic tissue. The spleen is readily separated from its attachments and splenectomy is easily performed with very little hemorrhage. Small nodules of accessory splenic tissue are not infrequent, and are usually found embedded in pancreatic tissue near the hilus. The anterior tip is occasionally bifurcate. Snell has described an anomaly in the shape of the spleen in a stock of mice now extinct. He described the shape as resembling an exclamation point.

The spleen undergoes great variation in size. This is to be expected when it is recalled that the white pulp is composed of a very labile lymphatic tissue and the red pulp actively participates in blood formation and varies in quantity depending upon the hematopoietic demand. Differences in weight are found depending upon strain and age, and to some degree upon sex. It is always important that spleen weights in an experimental group be compared with other animals of the same strain, age, sex, and environment in a control group. Measurements of spleens from adult strain C3H mice in this laboratory have approximated 15 to 20 $\times$ 4 mm., and the average weights for different control groups were from 106 to 139 mg. (Dunn *et al.*). Figures given by Kaplan, Nogareda and Brown for saline-injected controls of strain C57BL, 33 $\pm$ 3 days of age, ranged from about 80 to 100 mg. in males, and from about 70 to 90 in females. Santisteban in a doctoral thesis reporting work done in Dougherty's laboratory, using strain CBA mice, reported spleen weights at different ages as follows: Male, 74.5 $\pm$ 10.9 at 6 weeks, 110.4 $\pm$ 12.2 (the maximum for this group) at 26 weeks, and 87.8 $\pm$ 15.3 at 62 weeks. Female, 61.4

± 4.5 at 6 weeks, 110.6 ± 4.2 (the maximum) at 14 weeks, and 77.1 ± 4 at 62 weeks.

Spleen size is generally more uniform in young normal mice than in older mice. With food restriction or vitamin deficiency the spleen may be greatly reduced in size and weigh not over 10 milligrams (Dunn *et al.*). In leukemoid conditions the weight may reach 1.6 gm., and even larger sizes may be found in neoplastic diseases.

Morphology.—The type of circulation in the spleen, as demonstrated by *transillumination* methods in the living mouse, has been a controversial subject. Kniseley first observed the circulation in the spleen of the living mouse, and described the finer structure of the vascular system. MacKenzie *et al.* used a similar technique for transilluminating the spleen in a number of laboratory animals. The mouse was found to be best adapted for this method, and most of their conclusions were drawn from observations on this species. Contrary to Kniseley, they believed that an open type of circulation existed, and that the cells of the pulp came directly into contact with cells of the blood stream. Characteristic features of the spleen of the mouse were: relatively little capsular tissue, a sparse amount of muscle tissue, and weak rhythmical contractions; no structually distinct ellipsoids were seen, as in the cat, and no arterial anastomoses past the intralobular arteries, so that a sharply outlined infarction occurred when these were occluded. Active phagocytosis of erythrocytes was observed in the red pulp of the normal living spleen. The effect of various procedures was tried, and it was noted that: 1) ink was arrested in areas of the red pulp 20 seconds after injection into the tail vein, and within a few hours all particles were intracellular, where they might remain for several months, being retained in large fixed macrophages; 2) adrenalin caused arterial constriction and contraction of the organ; 3) hemorrhage caused contraction and the red pulp might be completely cleared of erythrocytes; and 4) starvation for 24 to 72 hours had a profound effect—the size of the spleen was reduced to ¼ to ½ of the normal, and transillumination became poor, probably due to the crowding together of pigment-containing cells.

Peck and Hoerr (1951*a*) repeated the observations of Kniseley and of MacKenzie *et al.*, with minor variations in technique to obviate some of the trauma and changes in temperature in the exteriorized spleen. They agreed essentially with Kniseley's conclusion that the blood passed through lined, intact, constant blood vessels which joined the arterial tree with the venous system, and had the peculiarity of leaking blood plasma, particulate matter, and even some blood cells to the extravascular spaces of the red pulp. They suggested that this special type of circulation might be designated as "separatory." Changes in environmental temperature (1951*b*) had profound effects. At 40.5° C. the storage phase was eliminated and the circulation became constant and rapid. At 37.5° C. the red blood storage activity was increased. Sudden thermal changes caused a strong initial contraction.

Histology.—Microscopically the spleen shows great variation. Extramedullary hematopoiesis is found so regularly in the red pulp that it should probably be considered a normal condition. The hematopoiesis may be predominantly granulocytic, and large numbers of early granulocytic forms are found next to the capsule and trabeculae, with more mature granulocytes in the central areas of the red pulp. A considerable number of eosinophils may be found, and megakaryocytes may be abundant. Erythropoietic activity is revealed by collections of nucleated red cells identified by the small, darkly staining nuclei. Depending upon the stimulus, the hematopoietic activity may be predominantly erythropoietic or granulopoietic, or a combination of both. Besides the blood-forming elements, the red pulp contains numerous fixed reticulum cells and macrophages, which often contain hemosiderin, especially in older mice. An apparent increase or decrease in hemosiderin within the red pulp may depend upon variations in the size of the spleen and a relative decrease or increase of the area of the red pulp. Relatively few blood-filled sinusoids are seen in the mouse spleen when compared with other species.

The white pulp is composed of lymphatic tissue which is similar to that found in other lymphatic organs, being composed of a reticular framework, reticulum cells, and lymphocytes forming lymphatic nodules. The splenic nodules vary in number and structure. Usually they show centers with a background of pale-staining reticulum cells among which there are a few lymphocytes, surrounded by a rim of numerous massed lymphocytes, which blend into the diffuse lymphatic tissue of the surrounding white pulp. The central artery is usually located toward the center of the nodule. The nature of the cells in the center of the nodule varies. They may be predominantly large pale cells, often containing chromatin debris, or predominantly large lymphocytic cells with basophilic cytoplasm and frequent mitotic figures, or cells of both these types may be found together. This variation in the character of the cells has caused disagreement regarding the function of the center. The first type may be referred to as a "reaction center" in which there is nuclear fragmentation of lymphocytes and ingestion by macrophages, and the second type as a "germinal center" in which new lymphocytes are forming. These centers are not fixed anatomical structures, but come and go, depending upon functional activity.

In the rat, a perifollicular envelope about the nodule was described by Krumbhaar (1948). He remarked that in the mouse spleen the "collar" was less well developed and more difficult to distinguish. In the rat this envelope is composed of a mononuclear type of cell which Krumbhaar identified as probably a young lymphocyte. Amyloid deposits, which are common in the mouse, often appear conspicuously in this zone (Dunn, 1944).

The lymphatic nodules in the spleen, the lymph nodes, and Peyer's patches of the mouse are less sharply outlined in man and many other mammals. A definite strain difference has been observed by Duqué, who

used a modified del Rio Hortega method for demonstrating the elements of the reticuloendothelial system in spleens from various inbred strains. Differences were demonstrated in the pattern of the reticulum and its arrangement in the nodule (figs. 8, 9, 10, 11). The arrangement in strain C3H (figs. 8, 9) showed a central area of the follicle surrounded by a well developed "perifollicular collar" of reticuloendothelial cells, one or two cell-layers thick. Peripheral to this collar there was a clear space, a "perifollicular halo" formed by reticuloendothelial cells in parallel rows. The numerous processes of these cells formed wide sinusoidal channels containing erythrocytes and lymphocytes. The reticuloendothelial cells in the red pulp were very numerous. Spleens from C57BL (fig. 10) showed more variation, both the perifollicular collar and the perifollicular halo being less sharply demarcated, and the perifollicular collar missing completely in some places. Spleens from strain A mice (fig. 11) showed even more variation in the development of the perifollicular collar, which in some animals was missing altogether.

The prussian-blue reaction was carried out in this laboratory on the spleens of mice of different strains at one year of age. A variation in the distribution of reticular macrophages containing hemosiderin was demonstrated in different strains. In strain BALB/c (fig. 12) a considerable amount of iron-positive substance was found in the red pulp, and very little in the nodules. In strain C58 (fig. 13) a relatively small amount of iron was found, and this was most abundant in masses in the center of the nodules.

Other strain differences have been observed. Strain A has a large number of mast cells, especially in old males, whereas mast cells are rare in strain BALB/c mice (Deringer and Dunn). Spleens of strain C57L mice examined in this laboratory showed less extramedullary hematopoiesis, fewer megakaryocytes, and a relatively smaller volume of red pulp than did the spleens of strain C3H and strain A. Kaplan (1952*b*) noted a difference in the histologic appearance of the spleen in strain A and strain C57BL examined following whole-body irradiation with the spleen shielded. Hematopoietic elements were more prominent in strain A. He suggested that the protective effect of spleen shielding might be genetically conditioned, and related to the importance of the spleen in the hematopoietic system of each strain. Stern reported that the storage of intraperitoneally injected carmine in Kupffer cells of the liver and in spleens from C3H and C57BL mice differed quantitatively and qualitatively. Strain C3H showed an inferior dye-storing ability. It is, therefore, desirable to work with an inbred strain if experiments are planned in which changes in the spleen may be significant. Histologic characteristics of the spleens of normal mice of the same strain and age should be compared with the spleens of the experimental animals.

The content of iron in the tissues generally increases in older mice. Blood formation, especially erythropoiesis, is very active in young healthy mice, and this activity frequently decreases with age.

An undetermined histologic lesion consisting of accumulations of small

cells with round, intensely basophilic nuclei is frequently observed in the reticular tissues of mice. The cells are of uniform size and structure and resemble mature lymphocytes except for their generally smaller size and greater intensity of staining. They lie within spaces or channels (fig. 14). This lesion has been seen in central areas of the white pulp of the spleen adjacent to the artery, in the medulla of the thymus gland, in lymph nodes, and in the outer portions of Peyer's patches. I observed the accumulation only once in sections from normal mice examined at 6 weeks and 1 year of age, but they were regularly seen in the lymphatic tissues of animals that had been exposed to bacterial toxins or nitrogen mustard several days before. No description of these formations in the mouse has been found, but Gillman and coworkers (1952) described a similar lesion in the lymph nodes of rats. They observed that the tightly packed lymphocytes seemed to be lying in lymphatic channels, but this appearance was considered to be an artefact and the space lying between the cords of cells and the adjacent pulp was considered to result from shrinkage. They believed that in actuality these cell masses were "highly lymphopoietic strands" of lymphatic tissue. Such an explanation does not appear satisfactory when applied to the lesion in the mouse, since an endothelial lining can usually be made out. Because this lesion has been found in recovery stages following stress reactions when many lymphocytes were developing and being discharged, it is suggested that they represent a congestion or stasis of lymphocytes, and not lymphopoiesis. Lesions of similar structure were also observed in reticular neoplasms (fig. 15). In the neoplastic tissue the enclosed cells may be either like normal lymphocytes or like the cells composing the neoplasm. Again it seems probable that these are trapped cells, which may have arisen from normal lymphatic tissue or from cells within neoplastic tissue.

2) Bone Marrow

Red marrow fills nearly all the bony cavities and there is little yellow marrow to be converted for hematopoiesis (fig. 16). Even in extreme hypoplasia, following food or vitamin restriction (fig. 17) or X-ray damage, the marrow seldom shows many adipose tissue cells. In normal mice very few hematopoietic cells are found in the peripheral two-thirds of the tail where the marrow is largely fatty (fig. 18), but even when hematopoiesis is very active, this space is not much utilized and other organs, particularly the spleen, compensate for activity in the marrow.

Morphology.—Sections from the sternum or vertebrae are recommended for microscopic study. In normal mice, cells in the femoral marrow are usually too crowded for microscopic detail to be visible. As noted earlier, the various immature blood cells are difficult to distinguish in the mouse and an attempt at the exact identification of early stages of development is not advisable. Granulocytes in marrow sections generally show a "ring" or "doughnut" nucleus. Nucleated red cells are recognized by their small, intensely basophilic nuclei. Megakaryocytes are prominent. Macrophages containing hemosiderin are frequent in old mice. Plasma

cells are not recognized in normal young mice and basophilic myelocytes are absent.

The foramina through which the vessels enter the vertebral bodies on the ventral surface, particularly in the lumbar area, are very large in the mouse, and marrow cells often extend through the foramina and are separated from the spinal canal by a thin membrane only. Immature marrow cells are thus found outside the marrow space, which may give a deceptive appearance of leukemic infiltration.

3) Thymus

Location and size.—The thymus is found in the upper mediastinum, overlying the base of the heart and the great vessels (fig. 7).

Like the spleen, the thymus shows great variation in size, so that control groups of mice should always be used for comparison; published "standard" weights are not a reliable comparison. In a group of strain C3H mice 50 days of age, examined in this laboratory, the thymus measured about 8 × 8 mm. in the broadest diameters, and the weights averaged 44 mg. for females with a range of 41 to 51 mg., and 37 mg. for males with a range of 33 to 44 mg. Figures given by Santisteban for male CBA mice were: 61.5±1.1 mg. (the maximum for the group) at 6 weeks, 18.7±1.9 mg. at 26 weeks, and 9.06±1.0 mg. (the minimum) at 62 weeks. Figures for females were: 66.5±2.4 mg. (the maximum) at 6 weeks, 32.5±28 mg. at 26 weeks, and 20±1.6 mg. (the minimum) at 62 weeks. Figures given by Kaplan, Nogareda, and Brown for immature (33±3 days) C57BL mice used as saline-injected controls in endocrine experiments ranged from 40 to 50 mg. for males, and 50 to 70 mg. for females. The sex difference in young mice is so pronounced that the authors advised separate statistical analyses of male and of female thymic weight data in acute experiments. The thymus does not undergo the same degree of involution with age as in man, and some thymus tissue can usually be found even in very old mice. It undergoes rapid involution, however, following food restriction, irradiation, and the action of certain "mitotic" poisons such as nitrogen mustard and podophyllin. Endocrine factors also cause changes, which will be discussed later.

Morphology.—Two lobes are found. The right lobe, which appears larger, overlaps the margin of the left. Microscopically, a cortex and a medulla are easily distinguished in young healthy mice but with food restriction, exposure to X rays or toxic agents, or after the action of certain hormones, the cortex may be greatly reduced in size and contain fewer lymphocytes than are found in the medulla—a reversal of the normal condition. Hassall's bodies are less well defined in the mouse than in man, and cannot be recognized in some strains. They are seen as indistinct aggregations of large, pale thymic reticulum cells which rarely form keratin in the centers. Small cysts usually located in the medulla and lined by cuboidal epithelium, often ciliated, are common in the thymus of the mouse. Thin-walled vessels in the medulla are sometimes found crowded with lymphocytes, a condition similar to that already

described in the spleen. The cortex of the thymus in young mice is rich in lymphocytes, and mitotic figures may be abundant. Andreasen (1948) counted the dividing cells in the thymus and compared the number of mitoses with those in the lymph nodes and spleen. The ratio in the thymus was about 0.3 percent, in the lymph nodes 0.06 percent, and 0.02 percent in the spleen. This demonstrated the importance of the thymus as a lymphocyte-producing organ in young mice.

There has been debate as to whether the small lymphoid cells of the thymus are identical with lymphocytes. Fichtelius summarized the evidence and stated, "So far no indisputable differences have been shown." Baillif injected rats with toxic acid colloidal substances, and observed the histologic changes that appeared during the induced involution and in the postinvolutional reconstruction of the thymus. He believed that the epithelial cells could transform into thymocytes, which were indistinguishable from lymphocytes. Phagocytes were a prominent feature in recovery stages. Lymphocytes from the thymus in the mouse appear to be somewhat larger and more deeply stained, yet they respond in the same way as lymphocytes to stress, and lymphocytic neoplasms originating in the thymus are morphologically indistinguishable from such neoplasms originating in the lymph nodes.

Changes in the thymus of the mouse related to age have been described in a series of papers by C. Smith and coworkers. Smith and Ireland prepared silver impregnations of the thymus from birth to old age, and noted an increase in argyrophil fibers, chiefly in the medulla. Smith and Thomas demonstrated glycogen by the leucofuchsin technique, and found it abundant in the small cortical cells shortly after birth and diminished thereafter, until at the age of 79 days only 1 or 2 cells giving a positive reaction were found in a microscopic field. The origin of the adipose cells during involution has also been investigated by Smith *et al.* (1952*a*). I have observed that macrophages with iron-positive pigment, such as are commonly found in the lymph nodes of older mice, are rare in the thymus. With the Giemsa stain, however, numerous distinctive, large, green-colored cells with one or more nuclei were found, most commonly just beneath the capsule, and the number of these cells increased with age. The unstained cell had a yellowish cytoplasm which the blue coloration of the Giemsa rendered green. Loewenthal and Smith carried out a series of histochemical tests on cells of this type, which they referred to as "lipid-laden foamy cells." The cells were strongly positive with the periodic acid–leucofuchsin method, even after diastase digestion. A structural similarity, but a cytochemical difference, was noted to the foam cells of certain human lipidoses. All the tests indicated the substance within the cells was a chromolipoid. The authors gave no specific name to these cells, but since they are sufficiently distinctive to warrant a name, "chromolipoid cell" might be adopted. Two other papers describe the changes in the vascular pattern during aging (Smith *et al.* 1952*b*) and the effects of irradiation on lipids in the thymus (Smith and Holst).

Mast cells may also be found in considerable numbers, even in young

mice. Increases after X-ray exposure and chemical carcinogens are discussed later.

Masses of epithelial cells resembling parathyroid tissue are sometimes found on the surface of the thymus, and this accessory tissue may be a reason why the mouse is so little affected by parathyroidectomy (unpublished observations of author). Conversely, the usual site of the parathyroid gland may sometimes be occupied by lymphatic tissue (fig. 19).

Considerable interest has been aroused by the possible role of the thymus in the genesis of lymphocytic leukemia in the mouse. Recent papers by Law (which are discussed later) indicate that the thymus has a more important role than merely furnishing an aggregation of potentially malignant lymphocytes.

4) Lymph Nodes

Names and location

The important lymph nodes in the rat have been located and described by Andreasen (1943), Andreasen and Gottlieb, and Job (1915). In general, the same nodes are to be found in the mouse. Both Andreasen and Job determined the drainage areas for the nodes in the rat by means of dye injections. Reports of similar investigations were not found for the mouse.

The location and general characteristics of the lymph nodes in the mouse are shown in figure 7 and described below.

Some variation has been noted in the size and number of nodes, both among individual mice and in different strains of mice. Large nodes that are usually single may often be found as two or more small discrete nodes. The following descriptions cover the usual conditions. In taking lymph nodes for microscopic section adjacent tissue can often be removed, and by this device many of the nodes can be identified without the need of separately labeled bottles. Whenever this device can be utilized, a notation is given following the description of the node.

Superficial cervical (fig. 20).—Two bilateral nodes lying above the salivary gland, the lateral often buried within the tissue of the parotid salivary gland. Frequent variation in number and in size, with enlargement especially when inflammation occurs about the face or head. May be removed with salivary-gland tissue for identification.

Deep cervical.—One or two small bilateral nodes, lying deep in the connective tissue between the sternocleidomastoid and the infrahyoid muscles, and anterior to the cervical plexus. Generally very small and difficult to locate in the healthy mouse.

Thoracic, mediastinal, or tracheobronchial.—Three or four small nodes lying behind the thymus in the adipose tissue near the bifurcation of the trachea. Often two small nodes on the right and a single larger node on the left. Sometimes greatly enlarged if there is inflammation in the lungs. May be removed with trachea or thymus.

Axillary (fig. 20).—A large bilateral node lying within the axillary fossa. Irregularly pear-shaped with a small protrusion pointing medially.

[Andreasen (1943) described this node in the rat as being composed of two fused nodes, one much larger than the other, and a similar process may account for the irregular shape of this node in the mouse.] May be removed with adjacent muscle for identification.

Brachial (*fig. 20*).—A large bilateral node, more dorsally situated than the axillary and lying superficially within the subcutaneous connective tissue, adjacent to the belly of the biceps. The scapular muscles have to be separated to reach it from a ventral approach. May be removed with a piece of skin.

Pancreatic or pyloric.—A pair of nodes attached at one margin to the edge of the pancreas, and separated from each other by a branch of a celiac vessel passing from the lesser curvature of the stomach to the hilus of the liver. Found by turning the stomach upward over the liver and following the course of the celiac vessel. May be removed with pancreatic tissue for identification.

Renal.—A bilateral node lying between the kidney and the aorta, the left cephalad to the renal vessels and the right partly obscured by the renal vessels which pass over it. Often difficult to find in healthy mice but frequently hyperplastic and particularly susceptible to pathologic alteration. Andreasen described it as a hemolymph node in the rat, but such alteration is not observed in the mouse. May be removed attached to the kidney.

Mesenteric[5] (*fig. 21*).—Usually a single elongated node lying in the mesentery of the ascending colon. Found by reflecting all loops of small intestine to the left. Occasionally smaller discrete nodes at either end of a single large node. Certain pathologic processes affect it primarily. Easily identified histologically because of characteristic architecture (described later) and large mesenteric blood vessels.

Inguinal.—A bilateral superficial node lying at the bifurcation of the superficial epigastric vein. Palpable in the groin and adherent to the skin. One of the largest and most easily accessible nodes, frequently selected for histologic study. In the female, mammary tissue can be removed with it for identification.

Lumbar (*fig. 22*).—A bilateral node generally lying above the bifurcation and on either side of the abdominal aorta. Node on left usually smaller and more caudally placed. May be removed with section of aorta and vena cava.

Caudal (*fig. 22*).—A single very small node lying below the bifurcation of the aorta. Usually removed with the lumbar nodes. (This node in the rat is reported by Andreasen and by Job to receive lymph directly from the skin of the tail.)

Sciatic (*fig. 23*).—A bilateral node lying in a fossa beneath the gluteal muscles slightly caudal to the point of emergence of the sciatic nerve. A small node difficult to find unless enlarged.

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[5] This node has sometimes been called "the pancreas Aselli" after Gaspar Asellius (1581–1622) who observed the lacteals in the dog and traced them to a group of nodes at the root of the mesentery, which had been called "pancreas." The term is now of historical interest only (Henry).

Other groups of nodes may become visible in leukemic mice, but those listed above can be located in healthy mice if examined with a dissecting microscope. Small nodes about the hilus of the liver, one in the lesser curvature of the stomach, and one in the popliteal space sometimes become visible.

MacDowell (1937) published a simple diagram representing the important lymphatic organs and nodes. It is less detailed than the diagram presented here, and offers a graphic, practicable method for recording the spread of neoplasms.

Weights

Santisteban recorded the weights of various lymph nodes in CBA mice as follows. Combined inguinal and axillary nodes for males were 15.8 ± 12 mg. at 6 weeks, 27.6 ± 2.4 mg. (the maximum for the group) at 14 weeks, and 22.5 ± 1.2 mg. at 62 weeks; for females—18.2 ± 0.8 mg. at 6 weeks, 24.2 ± 1.8 mg. (the maximum) at 26 weeks, and 16.7 ± 1.3 mg. at 62 weeks. Figures for the mesenteric nodes were: males—22.5 ± 1.2 mg. at 6 weeks, 23.0 ± 1.4 mg. (the maximum) at 14 weeks, and 8.7 ± 1.0 mg. at 62 weeks; females—21.1 ± 0.75 mg. at 6 weeks, 24.3 ± 1.2 mg. (the maximum) at 14 weeks, and 19.0 ± 3.7 mg. at 62 weeks.

Histology of lymph nodes in the mouse

The architecture varies with different groups of nodes. There are three general types, and the inguinal, the lumbar, and the mesenteric may serve as examples of the types. Since the inguinal node is readily accessible, and a good selection for routine observation, a microscopic description of this node is given, and variations from this pattern in other nodes indicated. The inguinal node is often incompletely divided into two parts (fig. 24). A marginal sinus lies beneath the capsule. Below the sinus is the cortical zone in which a variable number of lymphatic nodules are found, which are not so clearly outlined in the mouse as in many other species. Secondary centers may or may not be observed, depending upon the functional state of the node. The cortical zone is distinguished by a greater concentration of lymphocytes than is found in an adjacent wide "intermediate zone" of diffuse lymphatic tissue lying between it and the medulla. In young mice this intermediate zone is composed of recticular fibers and reticulum cells, and contains many lymphocytes. Lymphatic nodules do not develop in this zone, and there are few tissue spaces. In the inguinal node this zone may be separated into two parts by an extension of medullary tissue toward the cortex. In older mice the intermediate zone may be composed largely of plasma cells. The central area of the node is formed by the medulla, composed of medullary cords consisting of reticular fibers, reticulum cells, lymphocytes, and the medullary sinuses—endothelial-lined spaces in which a few monocytic cells are usually found. A notable feature of the lymph nodes of the mouse and the rat is the extraordinary height of the endothelium of the veins in the cortex and intermediate zone, which sometimes resembles, and has indeed

been mistaken for, a cuboidal epithelium. In pathologic nodes, spaces filled with lymphocytes may be found, such as were described in the spleen and other lymphatic tissue. Macrophages containing iron-positive pigment and mast cells are commonly found in the medullary sinuses of old mice. Mammary ducts are occasionally included in the hilus of the inguinal node in the female.

The lumbar node (fig. 25) differs from the inguinal node in having medullary tissue less centrally located, so that the cortical and intermediate-zone tissue may be found at opposite poles.

In the mesenteric node, cortical tissue and a relatively small amount of intermediate-zone type or diffuse lymphatic tissue lie along one border. The medullary sinuses are unusually wide and macrophages may be abundant. Extremely large, occasionally multinucleate cells, with vacuolated or pigmented cytoplasm, resembling the chromolipoid cells of the thymus, are frequent in old mice.

The lymph nodes occasionally show hematopoiesis, recognized by nucleated red cells, myelocytes, and megakaryocytes in the medulla. This alteration, however, develops only when there is an extreme demand for new blood cells, and is not a normal condition. Adipose cells are found in the hilus region of lymph nodes in old mice. This change has been seen most often in the tracheobronchial nodes.

The histology of the lymph nodes of rats has been described more fully than the nodes of mice, and some of these reports will be cited, since the information is often relevant to the mouse.

Kindred (1938) made a histologic study of the lymphoid organs of the white rat and described the mitotic and phagocytic activity in the lymphatic nodules. He distinguished a light and a dark zone, dependent upon the concentration of lymphocytes, in the secondary nodules. Separation of the secondary nodules into light and dark areas is less evident in the mouse, although occasionally seen.

Job (1922) thought the lymph nodes of rats could be separated into two general structural types, one in which the cortex was peripheral and generally surrounded the medulla, and a second in which cortical and medullary tissue were lodged at opposite poles and a septum ran partly across the node. Examples of the second type were the cervical, axillary, inguinal, and lumbar. Nodes in the mouse are not readily separated into these types.

Papers by Gillman and coworkers (1949, 1952) described the histology of lymph nodes in rats and emphasized the lability of the lymph-node structure. Nine different patterns were described. In contrast to the many variations in the rat, nodes examined in this laboratory from normal mice, aged 6 weeks and 1 year, have been remarkably uniform. In older mice with a variety of pathologic conditions nodes were found with relatively few or many lymphocytes, and with secondary centers showing either germinal or phagocytic activity predominantly. The morphologic variations in the lymph nodes of mice, therefore, may be less extreme than those in rats, and relatively young mice of inbred strains kept under

the best laboratory conditions have not exhibited the fluctuation observed by Gillman *et al.*

5) Peyer's Patches

The number of Peyer's patches in the small intestine vary somewhat depending upon strain (Kelsall, 1946). The highest number (around 11) were found in strain C3H, and the smallest number (6) in strain C57BL. Peyer's patches are prominent in mice and rats. They are usually located opposite the mesenteric attachment. Histologically Peyer's patches (fig. 26) contain lymphatic nodules, identical with those of the lymph nodes and spleen, and the centers in the nodules usually show the same physiologic state of activity as exists in other lymphatic organs. Intestinal villi are absent immediately above the nodule and the muscularis mucosa is interrupted at this point, so that the lymphatic tissue is separated from the lumen of the gut by a single layer of cuboidal epithelial cells, which may contain many degenerating leukocytes. The base of the villus at the margin of the Peyer's patch sometimes overhangs the edge of the intestinal epithelial surface, forming a cryptlike fold. The intestinal epithelium sometimes extends downward for a little distance into the lymphatic tissue, forming glandlike structures or evaginations of the mucosa. Iron-positive pigment is infrequent in Peyer's patches in comparison with the amount in lymph nodes and spleen.

6) Reticular Tissue of the Liver

Except for the Kupffer cells, no reticular tissue is ordinarily visible in the liver. The area about the portal vessels, however, is potentially a reticular tissue and extramedullary hematopoiesis is found frequently at this site. This area of the liver is also affected by many pathologic processes, which are described later.

IV. Non-neoplastic Alterations

A. Functional Response; Reaction to Stress and Hormonal Stimulation

1) Stress reactions and adrenal cortical hormone

The lymphatic tissues and blood-forming organs of the mouse undergo marked variation in size and cell population, a natural consequence of the responsiveness of lymphatic tissue to nutritional and hormonal states and the fluctuation in the demand for different types of blood cells.

Extramedullary hematopoiesis and a leukemoid reaction develop readily in the mouse, and present an important problem in differentiation from granulocytic leukemia. For this reason descriptions and discussion of this condition are included in the section on granulocytic leukemia.

A functional response that has been much studied in the last few years is the reaction of the reticular tissue to stress, or the "alarm reaction" of Selye. The most pronounced effects are on lymphatic organs, as reviewed by Dougherty.

The histologic features of the stress reaction have been induced by a variety of agents, among them cold, severe trauma, toxic chemicals, and bacterial agents or toxins. In intact mice the effect is mediated through the adrenal cortex, which is stimulated by the secretion of pituitary adrenotrophic hormone. Injection of adrenal cortical secretion reproduces these histologic changes, and removal of the pituitary gland or the adrenal gland prevents the reaction when a stimulus is applied. Dougherty and White (1945) described the changes in the mouse resulting from a single injection of ACTH. All the lymphatic tissues were affected, and the most severe destructive effects were found in the small and medium-sized lymphocytes (lymphocytolysis). A marked edema of the node occurred within 1 hour and persisted for 6 hours, during which time mitosis in lymphocytes ceased, the number was diminished, and degenerative alterations resulting in pyknosis and karyorrhexis developed. Later (6 to 9 hours) numerous macrophages filled with nuclear debris were observed, mitosis of lymphocytes was resumed, and numbers of histiocytes developed. A return to the normal pattern of the node required about 24 hours.

Santisteban gave detailed information on the weights of lymphatic (and some nonlymphatic) organs of intact CBA mice at intervals from 2 to 62 weeks, and on the weight response to adrenalectomy performed at different ages. The lymphatic organs attained the maximum weight before general body weight, then entered a period of involution in the following order: thymus, mesenteric nodes, peripheral nodes, and lastly the spleen. The weight of the thymus was significantly higher in the female. An increase in the absolute weight of all lymphatic organs followed adrenalectomy and was usually maximal at 5 days. The most pronounced effect of adrenalectomy was seen in the youngest and the oldest age group, but each lymph organ responded differently at different ages. The response of the thymus was greatest in the sexually mature animal. The adrenal gland weights were higher in the female and could be correlated with the greater relative weights of lymphatic organs in the female.

White reviewed and discussed the effect of endocrine secretions upon lymphoid tissue. He emphasized the general nature of the lymphocyte response to a variety of stimuli, including inanition and irradiation. He stated: "Any agent which can cause lymphoid tissue involution by a direct destruction of lymphocytes should mimic certain of the physiological consequences which result normally from the action of adrenal cortical steroids on one of their end cells, the lymphocyte."

Frank *et al.* studied the rates of involution and reconstitution of lymphatic tissue of both intact and adrenalized CBA mice following inanition and refeeding. The percentage loss of lymphatic tissue far exceeded the percentage loss of body weight during a 96-hour fast in the intact mouse. Adrenalectomy arrested the involution, and reconstitution occurred more rapidly in the operated group.

Other evidence of a relation between the adrenal glands and the number of circulating leukocytes, in different strains of mice, was presented by Stein and Cheng. Females from strains C3H, C57, and F were studied.

It was found that for these three strains an inverse relationship existed between the ratio of the adrenal cortex to body weight and the number of circulating leukocytes. Thus strain C57, with the highest total leukocyte count, had relatively the smallest amount of adrenal cortical tissue. An increase in total leukocytes developed after total adrenalectomy. Changes in the peripheral blood and bone marrow produced by massive doses of cortisone were described by Quittner *et al.* A prompt fall was noted in the level of circulating lymphocytes and eosinophils and the reduction persisted as long as 9 days. Granulocytopenia developed for 2 days, and was followed by a period of myeloid hyperplasia of the bone marrow. No inhibitory effect on cell proliferation in the bone marrow was observed, and there was no change in hemoglobin concentration.

The reduction in the number of eosinophils in the peripheral blood has been used as a test of the response to stress, pituitary and adrenal activity, and cortisone. References are given by Panzenhagen and Speirs who have described the effect of horse serum, adrenal hormones, and histamine on the number of eosinophils in the blood and peritoneal fluid of mice. They concluded that physiologic doses of cortisone, Compound F, epinephrine, or histamine do not have a direct lytic action upon the eosinophils. Kass *et al.* presented data which suggested that "white" mice might be unusually resistant to ACTH, since the injection of nonhormonal proteins gave as much effect as small doses of ACTH. Wragg and Speirs noted strain and sex differences in the response of inbred mice to adrenal cortical hormones. Females were considered generally less reliable. Some strains were highly sensitive, others relatively insensitive, and others too low in eosinophils to be practicable for testing. F_1 hybrid males from C57BR/cd $\times$ C57BL/6 were recommended for the assay described. Thompson and Blount investigated the age at which reactivity to stress first appeared in the mouse as indicated by eosinopenia, and found that newborn mice reacted to epinephrine and ACTH, but not to heat.

Prolonged stimulation of the adrenal cortex and adrenalectomy were reported to affect the numbers of circulating erythrocytes and the hemoglobin values as well as the lymphocytes (White and Dougherty). Daily injections of adrenotrophic hormone into mice resulted in an increase in hemoglobin and in red cells, an absolute lymphopenia, and an increase in polymorphonuclear leukocytes.

2) Other endocrine effects

The effects of other types of hormonal variation have been described. Andreasen (1948) reported a striking difference in the total amount of the organoid lymphoid tissue in male and female albino mice. This sex difference was most pronounced at 2 to 2½ months of age. The weights of the thymus, lymph nodes, and spleen were 2 to 3 times greater in the females.

Differences in the leukocyte count of male and female mice reported by Arvy (1944*a*) have been discussed. She also described changes during the estrus cycle in female mice. At the end of proestrus and the beginning

of estrus the granulocytes reached a peak and the bone marrow was very active. In the metestrus and diestrus periods small lymphocytes were most numerous and the granulocytes least numerous. The "leukogramme" of an ovariectomized mouse resembled that of an intact mouse at diestrus. Injection of estradiol, testosterone, and thyroxine increased the number of granulocytes and large lymphocytes. Desoxycorticosterone had no effect. In a later paper (1944*b*) the "leukogramme" of the male mouse was described and the effect of various hormones reported. Injections of testosterone caused an increase in monocytes but no other significant change. After orchidectomy anemia developed and leukopenia, in which the polynuclear cells were reduced, causing a relative increase in small lymphocytes. Testosterone injections in the castrate male caused a return to the intact male-type "leukogramme."

Marder reported that the administration of thyroxine elicited a statistically significant increase in the weights of the spleen, lymph nodes, adrenal glands, and kidneys, while no significant change occurred in the weight of the thymus.

Kaplan, Nogareda, and Brown presented a comprehensive review on endocrine factors and radiation-induced lymphoid tumors of mice. Work in this field is currently active and new information is rapidly being acquired. Results with different species of animals and different strains of mice are not always consistent, so that present information needs to be extended before general conclusions are reached. The review discussed pertinent published reports and gave information on work in progress in the authors' laboratory. The changes in weight in the thymus gland following the removal of different endocrine organs or the injection of hormones, and the late incidence of lymphoid tumors in the experimental mice are summarized in a table reproduced here (table 1). Removal of

TABLE 1.—*Comparison of endocrine effects on thymus and on lymphoid tumor development**

Endrocrine factor	Physiologic effect on thymus	Effect on lymphoid tumor incidence
Testosterone	Involution	Inhibition.
Orchidectomy	Hypertrophy	Augmentation.
Estrogen	Involution in large doses; possible cytologic aberrations.	Inducing agent in some strains; no effect in others.
Ovariectomy	Hypertrophy	No effect.
Adrenalectomy	Hypertrophy	Augmentation.
Cortisone, hydrocortisone	Involution	Inhibition.
Desoxycorticosterone	No effect in moderate doses; involution in large doses	No effect.
Hypophysectomy	No effect or relative hypertrophy in mice	No effect to date.
Growth hormone	Hypertrophy in rats; adrenal-mediated involution in mice	Inducing agent in rats.
Thyroxin	No effect in intact mice	No effect.
Hypothyroidism (thiourea)	Slight involution	No effect.

*After Kaplan, Nogareda, and Brown.

the testes, the ovaries, or the adrenals caused hypertrophy of the thymus. Cortisone, hydrocortisone, and relatively large doses of testosterone or estrogen caused involution. The effect of hypophysectomy and growth hormone was not clear-cut, and it was concluded that the growth and maintenance of the thymus and lymph nodes were not under direct pituitary control in the mouse. The correlation of these effects with the induction of lymphocytic leukemia is discussed in a later section.

B. Aging and Degenerative Changes

1) Aging.—Except for changes in the thymus and lymph nodes already discussed, no reports directly concerned with changes in the reticular tissue in the mouse due to aging have been found. Andrew and Andrew described age changes in the lymph nodes of Wistar rats. A progressive relative increase in tissue of the medulla was observed, but there was little fibrosis. The cortex sometimes contained nodular masses of plasma cells which were believed to have developed from lymphocytes. The average number of reaction centers decreased with senility while the number of large free macrophages in the medulla, and signs of phagocytic activity of these cells, increased with age. The capsule of the node showed a tendency to become broader in old age and there was separation of the fibers and infiltration by cells. Mast-cell accumulations were increased in some nodes and cystic areas were sometimes noted.

Much the same types of change have been observed in mice. Occasional nodes show small amounts of fat in the region of the hilus, and in the tracheobronchial node, fatty infiltration may be extreme. Fibrosis is rare. An increased number of mast cells is usually found in the hilus, and often an increase in iron-positive macrophages in the same area. The number of plasma cells also increases.

Many other degenerative and inflammatory changes, while not specifically related to aging, are more frequent in old mice.

2) Hemosiderosis.—Erythrophagia is sometimes a striking histologic feature in the sinuses of the lymph nodes and spleen. Erythrocytes are seen adhering to the surface of macrophages and within the cytoplasm. Although hemosiderosis is increasingly frequent with advancing age, young mice under pathologic conditions may show considerable amounts of iron-positive material, especially in macrophages of the spleen. An apparent increase develops when the size of the spleen is reduced for any cause, so that hemosiderin-containing macrophages are relatively increased. The different groups of lymph nodes vary considerably in some mice, indicating a reaction to local conditions. A generalized hemosiderosis accompanying hemolytic anemia was produced in this laboratory by giving mice toluidine blue in the drinking water.

3) Amyloidosis.—Amyloidosis is frequent in mice, and is often observed in the spleen. It may be a primary or idiopathic type as in strain A, where it is a strain characteristic (Heston and Deringer, 1948), or it may be secondary to a variety of conditions or procedures (Dunn, 1944).

Characteristically amyloid first infiltrates the perifollicular zone in the spleen and the hilus in lymph nodes.

4) Cyst formation.—Cysts filled with clear or slightly milky fluid, sometimes a half-centimeter or more in size, are occasionally seen in the lymph nodes. They are more frequent in old mice, and no cause for their formation has been determined. There is no associated inflammatory reaction and they seem to be due to dilatation of the lymph sinuses. The wall of the cyst is not fibrous and the surrounding lymphatic tissue appears normal.

5) "Mesenteric disease."—This designation was used by Simonds (1925*b*) for a condition found in 5 of 15,000 mice of the Slye stock. Characteristic features that he noted were: enlargement of the mesenteric node up to 4 to 10 times the normal size with little or no change in other organs; wide blood-filled spaces, with incomplete endothelial linings, which disrupted the normal histology of the node; and a decreased number of lymphocytes lying in a delicate reticulum between the spaces. In one mouse another group of nodes, the retroperitoneal, was also affected. The nature of the condition was not determined. It did not resemble a hemolymph node, and it had only a slight resemblance to hemangiomas such as were seen in the spleens of 2 or 3 other mice in the Slye stock.

The above description is the only reference I have found to this condition, yet it is seen frequently in old mice, particularly in strain C3H. The information I have is derived from random observations and a review of 60 microscopic specimens collected from old C3H males by Dr. Oscar Duqué. A few details can be added to Simonds' description. The medulla was usually affected before the cortex, and spaces filled with serum or free erythrocytes were surrounded by a meshwork composed of cords of spindle cells and leukocytes. Collections of plasma cells were often present in these cords in the medulla, and foci of granulocytopoiesis, often with a surprising number of mature granulocytes, were also found here. Little erythropoiesis was seen. Megakaryocytes in varying number were often found, usually lying within the blood-filled spaces of the medulla (fig. 27). Erythrophagia was frequent, and macrophages containing hemosiderin were numerous in some cases. The cortex in advanced cases also showed numerous large, round spaces filled with erythocytes (fig. 28). Secondary centers composed of large pale cells and a few small lymphocytes, but showing no phagocytic or mitotic activity, were found in a few nodules. Simonds' observation of atrophy of lymphatic tissue was confirmed.

Occasionally one portion of the node was more severely affected than remaining portions. Surprisingly little evidence of thrombosis, necrosis, or fibrosis was found. Dilatation of thin-walled vessels was often seen at the periphery of the node beyond the capsule, but sections of adjacent arteries were found to be of normal size with intact walls.

I have not attempted to correlate possible changes in other organs with the alterations in the mesenteric node. The lesion was usually confined to this node, although occasional involvement of the pancreatic

and other nodes was noted. Mesenteric disease was not directly related to neoplasia, although rarely nodes showing this lesion were also the site of hemangioendothelioma or reticulum-cell neoplasms. Venous congestion is probably the best explanation of the condition, but no site of interference with venous return was found. It is important to recognize the condition and not to confuse it with true neoplasia. It is noteworthy that it develops often in strain C3H and in hybrids with one strain C3H parent. Dr. Henry Kaplan observed it often in old C57BL mice (personal communication). This suggests a genetic basis.

C. Hyperplasia of Particular Cell Types

Enlargement of lymph nodes and other lymphatic tissues may result from hyperplasia of a single cell series.

1) Reticulum cells.—Similar reactions in the lymph nodes of man have been termed reticuloendothelial hyperplasia (Forbus) and histiocytic sinus reticulosis (Robb-Smith), and "sinus catarrh" by earlier pathologists. The lesion is especially frequent in old mice, and is often found in nodes draining an area of chronic inflammation. It may closely resemble a preneoplastic lesion described by Potter *et al.*, to be discussed later.

The reaction primarily affects the sinuses. The littoral cells are increased in number and their cytoplasm is increased in amount and is usually markedly eosinophilic. The cells may form sheets of fusiform cells which entirely occupy the sinuses (fig. 29) in a limited area, or the cells may be rounded, detached, and lie in dilated sinuses (fig. 30). Lymphocytes in the cortex may be reduced, and germinal centers are infrequent.

In the rat a similar reaction resulting from injections of trypan blue has been described by Gillman *et al.* (1952) and named "sinus histiocytosis." More advanced lesions of this type were referred to as "histiocytomas," a misleading term since histiocytoma is generally used to denote a neoplasm.

2) Plasma cells, including cells with Russell's bodies.—An increase in plasma cells is frequent in the lymph nodes of old mice and the nodes may be considerably enlarged. The plasma cells may overshadow all other cellular elements in the medulla (fig. 31) but lymphocytes still persist in the cortex, and lymphatic nodules can usually be recognized. The plasma cells are confined within the capsule of the node, a feature that distinguishes this lesion from plasma-cell neoplasms. Other reticular tissues and the perivascular area of different organs may contain plasma-cell accumulations. Parsons (1943) noted that a few plasma cells were normally found at the hilus of the lymph node, and she thought they were more abundant in the "submental" and the mesenteric nodes. An increased number was found in mice exposed to X ray, and treatment with 1:2:5:6-dibenzanthracene produced a similar effect. I have observed an increase of plasma cells in old mice such as was reported in the rat by Andrew and Andrew. Gillman *et al.* (1952) have designated

plasma-cell accumulations in the nodes of rats as "plasmacytoma," a term which suggests a neoplastic alteration.

Cells with Russell's bodies.—Rarely, lymph nodes are found which may be enlarged due to aggregations of plasma cells interspersed with a large number of cells with Russell's bodies, which appear to be developing from the plasma cell (fig. 32). This lesion is rarely seen and its nature is undetermined. Although the node may be considerably enlarged the lesion does not appear to be neoplastic. This change has usually been confined to a single node, although occasionally a number of organs of the reticular tissue may show an accumulation of plasma and Russell-body cells.

3) Mast cells.—Mast cells in lymph nodes are more frequent in old mice. They are usually discrete, and are often associated with hemosiderin-containing macrophages within the medullary sinuses near the hilus. Increases in mast cells following irradiation and exposure to chemical carcinogens are discussed in a later section.

D. Reactions to Injury

1) Unidentified irritants with manifestations of acute, chronic, or granulomatous inflammation

In reacting to various types of injury, the reticular system of the mouse displays many of the same histologic features found in man. A lymph node that drains an area of inflammation is usually enlarged and edematous. The cervical nodes furnish frequent examples of this response, which is secondary to acute and chronic inflammation in the oral or nasal cavities or in the skin about the head and face. In acute conditions the sinuses of the node are dilated and filled with albuminous fluid, in which many free macrophages, often with vacuolated cytoplasm, are found. The secondary centers may be prominent, and may show mitotic activity, phagocytic activity, or both. Many granulocytes may be present in the medulla, and abcesses are sometimes found. When there is extreme granulocytosis, foci of extramedullary granulocytopoiesis are usual in the medulla of the node, and should be distinguished from inflammatory granulocytic infiltration. If the inflammatory irritant persists, reticulum cells accumulate in greater numbers, and the condition of reticulum-cell hyperplasia develops. Lymph nodes draining areas of dermatitis in pigmented mice may contain masses of melanin, such as are found in lymph nodes draining areas of dermatitis in dark-skinned human beings.

Among the nonspecific inflammatory reactions, a chronic granulomatous process is occasionally encountered which resembles a conglomerate tuberculous process or sarcoidosis in man. Many epithelioid cells and Langhans-type giant cells are found (figs. 33, 34). The granulomatous tissue is often diffuse, without localized nodule formation or focal necrosis. Granulomatous areas of this type have been seen most frequently in the renal node. No specific etiology has been recognized, and attempts to detect an organism by special bacterial stains in sections after paraffin

embedding have been unsuccessful. This lesion has been sporadic, appearing rarely and in single mice from a variety of different groups. It is usually a localized process affecting only one node, though occasional mice have shown areas of similar granulomatous reaction in the spleen, the liver, and several nodes.

2) Living organisms

a) Known bacterial and viral agents.—Certain specific infections in the mouse are characterized by changes in the lymphatic organs. These have been reviewed by Dingle. One of the most commonly encountered is "mouse typhoid" caused by *Salmonella typhimurium*, which enters through the gastrointestinal tract and primarily invades the Peyer's patches at the lower end of the ileum and the mesenteric lymph node. On gross examination these structures are enlarged and are edematous. Ulceration of the mucosa covering the Peyer's patches may follow. On microscopic examination the mesenteric node and the spleen often show focal necrosis. The spleen is regularly enlarged to 3 or 4 times its normal size. Lesions in the lymphatic tissue are prominent in the early stages of the infection, and the liver lesions that suggested the term "liver disease" are a later development.

Ringlike piroplasmic bodies, *Eperythrozoon coccoides*, have been found on the red cells and in the serum of the peripheral blood in some groups of mice following splenectomy or X-ray exposure, when a latent infection was activated (Marmorston). We have not encountered this in blood smears examined at the National Cancer Institute.

Numerous reports are published of bacteriologic investigations in which mice were infected with organisms known to be pathogenic for man, but these are not reviewed here.

b) Unidentified agents.—Reactions of the lymphatic system in the mouse to unidentified filtrable agents which were transmitted along with leukemic tissue have been described. In three reports the filtrable agent affected the peripheral blood and the lymphatic tissue, and a modification in the biologic behavior of the leukemia line developed.

The agent described by Taylor and MacDowell caused hypertrophy of the lymph node and spleen, involution of the thymus, and moderately high white-blood-cell counts. Medium lymphocytes were the predominant cell in the lymph nodes and collections of eosinophils, plasma cells, and cells with Russell's bodies were notable. When an extremely small amount of the virus-infected line was used for transplantation no leukemia resulted, whereas nearly all animals injected with a similar amount of the virus-free line developed the disease.

Law and Dunn observed a moderate leukocytosis in mice injected with cells of a leukemia line contaminated by a filtrable self-propagating agent. Extreme reduction in the number of lymphocytes was found in the lymph nodes, thymus, and spleen, and hyaline necrosis developed in these organs and in the adipose connective tissue. Very little histologic evidence of

leukemia was found in nonresistant mice injected with cells of the contaminated line, while similarly injected mice that were previously given the cell-free filtrate, and thereby developed some resistance to the agent, showed the usual wide dissemination of leukemic cells.

De Bruyn (1949*a*) described a lymphopenia-causing agent transmitted with lymphosarcoma.

These reports demonstrate that the contamination of a transplantable leukemia line by filtrable agents may alter the hematologic response in the host, and the growth of the neoplasm may be modified. The possibility of contamination by an organism should always be considered when an unexplained change develops in a transplanted neoplasm.

A transmissible disease in mice characterized by anemia, leukopenia, and "erythropoietic splenomegaly" was described by Upton and Furth, who thought it was probably caused by an unidentified organism. It could be transmitted by the injection of particles of spleen from affected mice. The course was chronic, and 3 to 5 months elapsed between inoculation and onset of symptoms.

3) Mitotic poisons

A number of toxic drugs act like colchicine, producing an effect upon cells in mitosis. Among these are urethan, nitrogen mustard, and podophyllotoxin. The histologic effects are remarkably similar, and the lymphatic tissues are especially vulnerable. Clearkin described the effect of colchicine in the mouse. The cortex of the thymus and the red pulp of the spleen were altered, whereas the lymphatic nodules in the spleen were little affected. Graef and coworkers described the effect of nitrogen and sulfur mustards on mice and other laboratory animals, and Bass and Feigelson the effect of nitrogen mustard and urethan. All of these substances damaged the lymph nodes, the spleen, the bone marrow, and the epithelium of the small intestine. In the lymphatic tissues, the major effect was upon the lymphocytes, which were rapidly destroyed, and the chromatin debris was phagocytized by macrophages. The reticulum cells persisted. Hematopoietic tissue of the bone marrow was also severely injured, and became aplastic. Dr. Henry Kaplan stated that mustard (HN_2) had little effect on thymic or peripheral lymph-node weight in C57BL mice when given at sublethal doses (personal communication).

Some of the lymphocyte-damaging effect of these drugs may be mediated by hormones of the adrenal cortex.

4) Irradiation

The changes produced by the mitotic poisons are so like changes caused by X ray that they have been called radiomimetic drugs. Destruction of lymphocytes is an outstanding feature in both, but Koller and Casarini have concluded that the primary basic reactions initiated by X rays and nitrogen mustard are fundamentally different, and that cells at the end

of the resting stage are most sensitive to X rays and least sensitive to nitrogen mustard. Barrow and Tullis described X-ray effects in mice and emphasized the similarity of lesions in all lymphatic tissues, whether in the spleen, lymph nodes, or Peyer's patches. The reticulum cells and fibrils appeared undamaged. Erythropoietic tissue was extremely sensitive, and erythrophagocytosis was marked. Plasma cells appeared undamaged and even seemed to increase after irradiation. Brecher *et al.* (1948), using a smaller dose of irradiation, observed the changes in the blood and blood-forming organs for a longer period, and reported no morphologic evidence of damage to the reticulum cells, whereas the lymphoid, myeloid, and erythroid cells were markedly injured. Spargo *et al.* described the histologic effects of long-continued, whole-body, gamma irradiation of hybrid LAF_1 mice. The hematopoietic organs, with the exception of the lymph nodes, underwent progressive depletion, and at late intervals erythropoiesis increased in the spleen. An increase in mast cells was reported.

Warren *et al.* reported the effects of internal irradiation of mice with P^{32}. The degree of injury and regeneration varied in different reticuloendothelial and hematopoietic tissues. The thymus showed earliest damage and quickest recovery. Spleen and bone marrow appeared about equally sensitive, but bone-marrow recovery was later, no doubt due to P^{32} retention in the bones. Lymph nodes were the last to show morphologic recovery. A later "overgrowth" effect appeared in some tissues, but not in lymph nodes, after a preliminary "depressive" effect.

The subject of irradiation in mice has been of special interest since the report by Jacobson *et al.* that by shielding the exteriorized spleen, survival after lethal dosage of whole-body irradiation resulted. Experiments in a number of laboratories on the effect of different procedures and the injection of different substances suggested that a humoral factor or factors was concerned in the recovery. The data were summarized and discussed by Jacobson in a review. Congdon *et al.* made a histopathologic study of mice and guinea pigs injected with bone marrow following acute irradiation, and concluded that the destructive action of X irradiation on hematopoietic tissues was unaltered, but that regeneration of these tissues was greatly enhanced and was responsible for the recovery. Of great significance is the lessened incidence of radiation-induced lymphocytic neoplasms, to be discussed later.

5) Growth of tumors

Profound effects on the lymphatic and hematopoietic tissue in mice have been reported following the growth of tumors. Considerable specificity has been recognized and some tumors elicit a characteristic response. Hueper (1934*a*) reported megakaryocytosis and leukemoid reactions (1934*b*) in white mice with spontaneous mammary tumors. A leukemoid reaction accompanied the growth of a spindle-cell sarcoma (Parsons 1935), an anaplastic sarcoma (Lewis 1937), and several lines of myoepithelioma and a sarcoma (Bateman). I have seen extreme erythrocytopoiesis in

mice bearing reticulum-cell sarcomas. Parsons (1943) described a "plasmocytoid" reaction in the lymphatic tissue of mice bearing either primary or grafted tumors. She suggested that the fixed reticulum cells proliferating in the lymph nodes were the precursors of the plasma cells.

V. Neoplastic and Related Conditions

A. Terminology

The terminology of neoplastic diseases of the reticular system in the mouse shares in (and is indeed a reflection of) the confusion that has beset the naming of these conditions in man. An attempt to resolve the chaos in human pathology was made by the Committee for Clarification of Nomenclature of Cells and Diseases of the Blood and Blood-Forming Organs. It is desirable that the terminology recommended by this Committee for neoplasms in man should be applied as closely as possible to similar forms of neoplasms seen in animals. While it is true that the pathologic processes are not identical, there is enough equivalence to make transposition of many terms feasible. Moreover, the normal cells in each species are equivalent and it is from the normal cells that the terminology of the neoplastic alterations is derived when a histogenetic classification is used. Neoplasms of the reticular system in the mouse are generally recognized as originating from stem cells, granulocytes, lymphocytes, reticulum cells, and plasmacytes.

Earlier reports of neoplasms of the lymphatic tissues in mice often attempted to classify the lesions according to the criteria current in human pathology. Simonds (1925*b*) for example, separated his cases of neoplasms of lymphocytes into leukemia, pseudoleukemia, subleukemic leukemia, leukosarcoma, and lymphosarcoma, which were distinctions commonly used in human pathology at that time. Such distinctions later proved difficult to apply in both human and animal pathology for actually these terms all indicated a malignant change in a single cell form, and whether the malignant cell remained confined in a localized growth or was dispersed in the blood stream often depended upon the time and the thoroughness of the examination. Richter and MacDowell (1935) proposed that, since these were all "varieties of the same fundamental condition differing in the distribution of lesions," the term "leukemia" would be used in reference to all these conditions, "including the myeloid forms, but not Hodgkin's disease." This is a practical suggestion which most workers dealing with neoplasms in mice have adopted. The term "leukemia" therefore has become an inclusive term for many of these malignant conditions irrespective of whether or not malignant cells were found in the peripheral blood. In this laboratory we often add the term "localized" or "generalized," depending upon whether the growth remained for some time as a localized tumor, with relatively few leukemic cells in the peripheral blood, or whether a widespread dissemination with numerous leukemic cells in the peripheral blood was found while the local tumor remained relatively small.

Regardless of present practice, we have inherited a long list of terms from preceding publications, and in the following descriptions of the different types of neoplasms a list of previously used terms is given.

B. Classification

The pathologist must sometimes wonder whether a histogenetic classification of tumors may not obscure more than it illuminates. Such a classification is based on obvious similarities in morphology to the presumed cell of origin. Strict uniformity is seldom found in the morphologic characters of tumors even when they appear to have the same derivation. This fact, added to the differences in interpretation which different pathologists may make of the same morphologic feature, are reasons why the application of a classification based on morphologic features is often inconsistent. Most classifications tend to minimize differences and exaggerate similarities. Where etiology can be determined, and a separation of diseases made on the basis of etiology, more exact classification is possible. Nevertheless, classifications are often required as a form of shorthand so that a considerable quantity of data can be presented in an abbreviated form, thus obviating the necessity of individual descriptions of many similar lesions. Classifications and tabulations of different types of tumors may also reveal relations to other important conditions in tumor-bearing animals, such as age, sex and strain, which might be overlooked if they were either considered individually, or all lumped together.

A number of other difficulties cannot be avoided in any attempt to apply a histogenetic separation to groups of tumors. Initially, there is the difficulty of deciding that the lesion is neoplastic, and this decision can be as uncertain in lesions of the mouse as in lesions of man. The same morphologic criteria are applied in both species. There is this advantage in the mouse, that if a new growth can be produced by the transfer of living cells and by this method only, an indubitable proof of malignancy is presented, and morphologic criteria of malignancy can be interpreted with more assurance.

Secondly, study of many transmitted lines of leukemia in the mouse has often shown that under similar controlled conditions each of these neoplasms has one or more features which are individual to that neoplasm and by which it is distinguished from all other neoplasms arising from blood cells. The distinctive features may be the biologic behavior of the leukemia line, or slight morphologic differences, or frequently both. Awareness of these differences is lost when the tumor is fitted into a general category. This is an unfortunate effect of classification, for it gives a misleading impression of uniformity.

A third difficulty is that the morphologic characters that are distinctive of one type of neoplasm may gradually merge into the characters of another type, through a series of borderline tumors that are not typical of either group. To make a separation is like trying to place a point between black and white, when they merge through a series of grays. Yet setting aside a category of gray seldom solves the problem, for two points of indecision are now introduced. For this reason the placing of

borderline tumors in one group or another often becomes an arbitrary decision and a source of error in applying a classification. Fortunately, the percentage of these borderline tumors is usually small.

Still another difficulty arises when the material available to the pathologist is inadequate. More facts on the biologic behavior of the tumor, well-fixed tissue, a greater number of histologic sections, special technical procedures, and a study of the peripheral blood would often permit a positive diagnosis when an equivocal one must be given.

Past experience has shown that in any large group some tumors are always found that must be put into an unclassified or uncertain category. For example, Richter and MacDowell (1935) reported that the type of leukemia could not be determined in 87 of 543 cases, in most instances because of post-mortem degeneration; Brues and Marble diagnosed "early or questionable" leukemia in 10 percent of both their experimental and control mice; Hill classified 9 cases as atypical, and 23 as mixed in a group of 134 tumors; and Simonds (1925*b*) noted that "borderline or transition cases were numerous," and in a number of his mice there was a difference of opinion among pathologists as to the diagnosis. For this reason, in every large collection of neoplasms it is usually necessary to give individual descriptions of a few tumors that do not fit into the categories provided, and that appear to be unique. It is also important to remember that with so many variables no classification of tumors can be absolute, and the pathologist makes his interpretation on the basis of the material available and his past experience.

With these reservations in mind, the following classification for neoplasms of the reticular tissue of the mouse is presented. It has been used in this laboratory and is the most satisfactory tested so far, considering the limitations in our present state of knowledge.

Classification of neoplastic and related conditions of the reticular system of the mouse

Cell of origin	*Neoplastic condition*
1) Undifferentiated	Stem-cell leukemia
2) Lymphocyte	Lymphocytic neoplasm—localized (lymphosarcoma)
	Lymphocytic neoplasm—generalized (lymphocytic leukemia)
3) Granulocyte [6]	Granulocytic leukemia (rarely a chloroleukemia)
4) Reticulum cell	*Type A*
	Reticulum-cell sarcoma—localized
	Monocytic leukemia—generalized
	Type B
	Hodgkin's-like lesion
	Type C
	Potter lesion—(doubtfully neoplastic)
5) Plasmacyte	Plasmacytoma—localized
	Plasmacytic leukemia—generalized
6) Tissue mast cell	Mastocytoma—localized
	Mast-cell leukemia—generalized
7) Miscellaneous and unclassified	

[6] Extramedullary hematopoiesis is described in comparison with granulocytic neoplasms.

The absence of any report in the mouse of certain neoplastic diseases found in man and originating in cells of the reticular tissue should be noted. Since the mouse does not have a blood basophil, a true basophilic granulocytic leukemia does not exist. Only one case of eosinophilic granulocytic leukemia has been reported. Neoplasms of megakaryocytes or thrombocytes have not been recognized, and polycythemia vera has not been reported.

For each type of neoplasm listed in the classification, the following items are considered: *a*) terminology, *b*) previous descriptions, *c*) characteristic case (in which my own experience and that of others is summarized), *d*) differential diagnosis, and *e*) comparative pathology (in mammals). A general discussion is also given of each type.

C. Neoplasms

1) Stem-Cell Leukemia

a) *Terminology.*—In human pathology this term refers to cases of acute leukemia in which the neoplastic cell is undifferentiated and has no distinguishing features of the granulocytic, lymphocytic, or monocytic series. Other terms in human pathology are: hemoblastic, hemocytoblastic, lymphoidocytic, embryonal or undifferentiated leukemia (Wintrobe). "Stem cell" applied to leukemias in mice has much the same usage, indicating a neoplasm of cells that cannot be identified as granulocytic or lymphocytic. Synonyms in the mouse are: undifferentiated leukemia, primitive-cell leukemia.

b) *Previous descriptions.*—Engelbreth-Holm in discussing "lymphogenous leukemia" described cases composed of "slightly larger cells with pronounced basophil non-granular protoplasm and relatively large round or often slightly irregular, leptochromatic nuclei containing from 2–5 nucleoli." These cells did not bear any resemblance to lymphocytes, but transitional forms between these cells and typical lymphocytes were found in some cases, and the author thought that classification as lymphoblasts was probably justified. In other cases cells were found that were closely similar to lymphoblasts morphologically, but they occasionally showed a positive peroxidase reaction indicating a myelocytic-cell type. Engelbreth-Holm, therefore, questioned whether such cases were to be regarded as derived from a stem cell that could undergo either lymphocytic or myelocytic differentiation, or whether they were "lymphoblastic" or "myeloblastic" but too undifferentiated to be distinguished from each other.

Kirschbaum and Strong noted that the stem cells of lymphatic and myelogenous leukemia might be quite similar.

Rask-Nielsen and Gormsen referred to the difficulty of distinguishing lymphoblasts from myeloblasts, and stated that it had lately been their practice to term leukemias showing these primitive-cell forms merely stem-cell leukemias. In their experience all the observed cases of leukemia in strain Street mice, except for the plasma-cell neoplasm, were stem cell.

c) *Characteristic case.*—Some cases of leukemia which we customarily

classify with lymphocytic leukemia have cells that are large with pale cytoplasm and large nuclei, sometimes slightly indented. The cell differs from the predominant cell types found in typical lymphocytic leukemia of the mouse. The cytoplasm contains no distinctive granules, and may sometimes be vacuolated. Leukemic lines in which this undifferentiated cell predominates grow rapidly and the cells are early disseminated in the blood stream.

d) *Differential diagnosis.*—This form of leukemia is distinguished from granulocytic leukemia by the failure of peroxidase treatment to reveal positive granules. It closely resembles less differentiated types of lymphocytic leukemia, or "lymphoblastic leukemia." In any large group of lymphatic tumors, types representing a transition from a highly differentiated lymphocytic leukemia with cells closely resembling a normal lymphocyte, to types of leukemia composed of large cells resembling an undifferentiated stem cell, are usually found. If the peripheral blood in the living mouse has been studied, and complete autopsies performed on freshly killed animals with such procedures as special stains and tissue smears, and if a transplanted leukemia line has been established and studied, the diagnosis "stem-cell leukemia" is justified, and should probably be used more often.

e) *Comparative pathology and discussion.*—The diagnosis of stem-cell leukemia in the mouse has not been used in many laboratories, and neoplasms of this type have usually been designated lymphocytic or lymphoblastic leukemia. The information required to separate this type of leukemia accurately from the granulocytic or lymphocytic forms is not always obtainable. With more study of leukemia in the mouse the diagnosis of stem-cell leukemia may be made oftener in the future.

"Stem-cell" leukemia in man is found among cases that run an acute clinical course. No report on the diagnosis of this form of leukemia has been found in other animals.

2) *Lymphocytic Neoplasms*

a) *Terminology*

The term lymphocytic neoplasm is recommended, to conform with present usage in human pathology. When the growth remains localized for a long period before leukemic cells appear in the peripheral blood, either the term "lymphocytic neoplasm—localized," or lymphosarcoma, is used to distinguish it from a type in which leukemic cells rapidly invade the peripheral blood and the local growth is small or absent, which is designated as "lymphocytic neoplasm—generalized," or lymphocytic leukemia. In some strains of mice the lymphocytic neoplasms often originate in the thymus and have been referred to as "thymomas," a term to be avoided, since true thymomas are extremely rare in mice.[7] The term lymphosarcoma of the thymus, or lymphocytic leukemia originating in the thymus, is suggested.

[7] I have seen only one case of a true thymoma in a mouse (figs. 72, 73), of strain 1194, autopsied by Dr. M. K. Deringer. Tumors of this type have been described in rats by Bullock and Curtis.

Other terms for the lymphocytic neoplasms have been: round-cell sarcoma, lymphosarcomatosis, leucosarcomatosis, lymphoma, lymphadenomatosis, lymphomatosis, lymphoblastoma, lymphatic leukemia, lymphogenous leukemia, lymphoid leukemia, lymphadenoma, malignant lymphoma, and (when the thymus was primarily involved) lymphosarcoma of Kundrat, and thymoma. Leukosis has been used as an inclusive term for both lymphocytic and granulocytic types of neoplasm.

b) Previous descriptions

Since lymphocytic leukemia is the most frequent form of leukemia in mice, and since it is the type that appears to be most directly related to genetic factors and therefore most favorable for controlled laboratory studies, the papers describing it are numerous and a comprehensive review is not attempted. The papers selected for reference are those which have reviewed previous publications extensively, or dealt especially with morphology, or made significant contributions regarding the nature of the disease. The papers are roughly divided into (1) early reports, (2) transmitted lines, (3) induced leukemia, (4) leukemia originating in the thymus, (5) cytologic studies, and (6) behavior in tissue culture. Since the present paper is primarily concerned with morphology, details of structure are emphasized.

(*1*) *Early reports.*—The earliest report of leukemia in a mouse was by Eberth in 1878. He noted an enormous spleen and no increase in size of the lymph nodes. Histologically the spleen and liver were infiltrated with leukemic cells resembling lymphocytes. Haaland in 1905 gave the best of the early descriptions, noting the excessive hyperplasia of both the visceral and the peripheral lymph nodes, mediastinal tumor, and leukemic infiltration in the liver, kidney and spleen.

Simonds (1925*b*), Andersen, Opie, Engelbreth-Holm, Emile-Weil and Bousser, Kaalund-Jørgensen (1936), and Krebs, Rask-Nielsen and Wagner have all made comprehensive reviews of the early work on leukemia, much of which consisted of case reports on a small number of animals with leukemia, usually of the lymphocytic type. Simonds (1925*b*) also described a number of cases from the Slye stock of mice. He diagnosed 28 cases of lymphatic leukemia and 29 of the myeloid type. Enlargement of the lymph nodes and spleen was always observed in the lymphatic leukemias, due to a marked proliferation of lymphoid cells, which usually were of the large lymphocyte variety. Invasion of the capsule by lymphoid cells was constant. The splenic pulp was extensively infiltrated, and mitotic figures were numerous. Lungs, liver, and kidney might show perivascular infiltrations and intracapillary leukemic cells. Simonds divided the lymphocytic neoplasms into a number of groups as follows: 1) sarcoma showing considerable local invasion, but no widespread infiltration, and corresponding to Sternberg's leukosarcoma in man, in 5 mice; 2) "pseudoleukemia," characterized by the histologic features of leukemia, but without invasion of the blood stream, in 111 mice; 3) lymphosarcoma, a sarcomatous process affecting lymph nodes or thymus exclusively,

without infiltration of other organs or blood-stream invasion, in 51 mice. The primary lymphosarcoma was intrathoracic in 32 mice, intraabdominal in 8, subcutaneous in 9, and too generalized to determine the original site in 2. Metastasis by way of the lymphatics was recognized in some cases. The kidneys and lungs were sometimes extensively involved. Generalized lymphosarcoma was admittedly difficult to differentiate from pseudoleukemia, since leukemic cells in both conditions possessed marked invasive powers, growing through the walls of the veins and the capsules of lymph glands and infiltrating the surrounding tissues. In both forms widespread involvement of the lymph glands of the body developed with infiltration of one or more of the viscera. The number of nucleated cells in the blood was higher in pseudoleukemia.

In addition to a comprehensive review of previous reports, Andersen gave detailed descriptions from his own material. He repeated the work of Heidenhain and injected heat autolysates obtained from human cancer and tumors of mice into several thousand mice. There was no appreciable difference in the incidence in control and experimental groups, however, and Andersen concluded that the neoplasms he described were probably spontaneous. He referred to leukemia in mice as "lymphomatosis infiltrans," manifested by a hyperplasia of one or more or all of the lymph glands, infiltration of the liver, the kidneys, the spleen, the bone marrow, and occasionally a great increase in the number of leukocytes in the peripheral blood. About one-eighth of his cases showed widespread involvement, about one-fourth were localized, and the remaining cases fell between these extremes. Andersen described in detail the relative involvement of different organs on gross examination. He separated his cases into 4 types: 1) homogeneous round cells with small amounts of eccentrically placed cytoplasm; 2) mixed forms; 3) forms with abundant cytoplasm and ring-formed or lobed nuclei, with an increase in megakaryocytes; and 4) special forms. When his descriptions and photographs are analyzed in the light of present knowledge, the first type probably represents lymphocytic leukemia, the second a miscellaneous group, the third granulocytic leukemia or extramedullary granulocytopoiesis, and the fourth proliferation of reticulum cells, either hyperplastic or neoplastic. Andersen considered that his classification was of relative value only, since in his opinion no sharp limitation was possible. He believed that the mouse might react in either a lymphatic or a myeloid direction, or sometimes both, and these reactions were different forms and gradations of the same disease which could not be sharply differentiated. It is probable that Andersen failed to separate leukemoid reactions so common in mice from the neoplastic disease, and this is one reason for confusion.

Andersen believed that it was incorrect to carry over the terminology of pathologic conditions from man to mouse, since the mouse was not a "little man," but a very complicated and sensitive experimental animal. There are arguments in favor of this point of view, but the terminology would become more hopelessly complicated than it now is, if a different

term from the one used in human pathology had to be invented for every neoplasm in every species of animal.

Engelbreth-Holm pointed out that relatively mature though somewhat atypical small lymphocytes characterized most cases of lymphogenous leukemia. In other cases larger cells, which did not bear a close resemblance to lymphocytes, were the predominant cell. These cells were usually lymphoblasts, although similar cells occasionally gave a peroxidase reaction, and were regarded as myelogenous.

(*2*) *Transmitted lines.*—It is remarkable that transmitted lines of leukemia in mice were not available until 1929, although the first mammary tumor in a mouse was successfully transplanted in 1898. Many attempts were made to transplant leukemia, sometimes using hundreds of animals for a single trial, but it was not until inbred animals were used by Richter and MacDowell (1929) in this country that success was attained. Korteweg in Holland reported success a few months later. Richter and MacDowell's transplants were made in strain C58, an inbred strain with a high incidence of leukemia, which appeared to be lymphocytic. Korteweg regarded his tumor as an example of Sternberg's leukosarcomatosis in the mouse, and there was uncertainty as to whether it might not have arisen in the reticuloendothelial system, but on injection of lithium carmine the tumor cells were pigment free. The tumor cells were described, however, as having the ability to phagocytize red blood cells. Transplantation was successful in about 24 percent of the trials.

Krebs, Rask-Nielsen and Wagner also succeeded a short time later in transferring lymphoid tumors that developed in irradiated mice into other irradiated mice. They noted that their cases closely resembled those of Korteweg. The tumor cells were thought to resemble lymphoblasts, and had an abundant strongly basophilic cytoplasm, sometimes with slightly eosinophilic granules with an oxidase-negative reaction. The nuclei were perfectly spherical. A detailed description was given of the infiltration in the organs and the peripheral blood. Various manifestations of the leukemia lines during transplantation were described. The transplanted mice presented "such a wealth of different pictures" that "leucemic lymphadenosis, aleucemic lymphadenosis, Kundrats' lymphosarcomatosis, and Sternberg's leucosarcomatosis" and "nearly every form of transition besides" were found. Since all these manifestations developed from the same identical tumor strain, the authors concluded that they all represented a single disease, and the name "lymphomatosis infiltrans (leucemica et aleucemica)" was suggested. The frequent origin of the tumors in the thymus was noted. The conclusion was reached that the implanted tissue caused the development of some toxic substance which provoked a pathologic growth of those tissues capable of producing lymphocytes. Protocols of a number of individual mice were given in an appendix.

This paper by Krebs *et al.* is remarkable because the leukemic lines failed to show an individual and recurring pattern of organ involvement such as was described in later reports. It may be that the lack of inbred

strains of mice and the necessity of using irradiated animals accounted for the variation.

The uncertainty as to the identification of reticular neoplasms in mice is well illustrated by the different opinions reached regarding a transmissible line of Krebs *et al.* Kaalund-Jørgensen (1936) made numerous experiments on its transmission, and studied its histologic features. He concluded that the changes in the blood and organs were "of a myeloid nature, and intimately connected with the reticulum." Further discussion will be found in the section on granulocytic leukemia.

After transmissible lines of leukemia became available, a number of morphologic studies of different lines were reported. Richter and MacDowell (1930*a*) described spontaneous leukemia in strain C58. During life, the most characteristic symptoms were: enlargement of the spleen, emaciation, labored breathing resulting from an intrathoracic tumor, and frequently an elevated leukocyte count. Abnormal cells found in the peripheral blood were larger than the normal mouse lymphocyte. The nuclei of the cells were large, usually round, and leptochromatic, and the network of chromatin demonstrated by the Romanowsky method was less dense than in normal lymphocytes. The cytoplasm was basophilic and frequently contained small clear areas near the nucleus, or small vacuoles. Specific granules were not found, but azure granules were occasionally present. Many cells appeared to be intermediate between an immature form and the normal lymphocyte. Microscopically, the same cells were usually found in the spleen, the liver, and the lymph nodes, and often involved other organs, such as the kidneys and pancreas. Four lines of leukemia were compared in a later paper (1930*b*). Different types of response were observed according to the line of leukemia inoculated, and the different lines were characterized by differences in the extent or distribution of leukemic cells, the total leukocyte count, the development of peritoneal and pleural effusion, and infiltration of the liver, kidneys, or other organs. With continued transplantation, the leukemic lines became more "virulent" and killed in a shorter interval of time in successive generations. An explanation of this phenomenon was searched for and an attempt made to relate it to morphology. Richter and MacDowell (1935) measured the cells in the infiltrations during periods of abrupt changes in the interval between transplantation and death and divided them into four classes. They found that the proportion of cells of each class changed gradually, and preceded the change in rapidity of growth. When the proportion of large cells increased, a decrease in the interval followed and *vice versa*. In a later paper MacDowell and coworkers (1937) stated that differences existed between the populations of leukemic cells found in spontaneous cases and the populations in the same neoplasm after transplantation through a long series of hosts. Different lines arrived at different degrees of cell maturity, and lines showing least differentiation were the most virulent. It was proposed that a change in the rate of differentiation, and not absence of differentiation, characterized leukemia; malignancy should be considered a modification of the rate of

normal differentiation, and not a new "species" of cells produced by some "saltation."

Cytologic studies in leukemia of strain C58 mice are discussed later.

Furth and associates have also described transmissible lymphoid leukemias. In a paper with Strumia, enlargement of lymph nodes, the spleen, and the lymphatic tissue of the intestinal tract was described. Microscopically, the organs involved showed a highly invasive neoplastic growth composed of medium and large cells resembling lymphocytes, many in mitotic division. In another paper, Furth *et al.* described a series of studies on "lymphomatosis" in mice. They noted, as did MacDowell and associates, that almost every line of transmissible leukemia differed from any other, and retained certain characteristic properties during transplantation. The predilection for certain organs was particularly distinctive. One line invaded the kidney, another the ovary, and another dorsal muscles and the spinal cord. One strain, S27, often produced thrombosis of small arteries in the lung, a characteristic of other lines of lymphocytic leukemia reported by Krebs *et al.*, Law and Dunn, Shelton, and de Bruyn *et al.* As in MacDowell's lines, the "incubation period" was reduced after repeated passages. It was postulated that this might be associated with some selection of the more rapidly growing or more resistant cellular offspring. It was concluded that leukemic lymphocytes were not lymphoblasts, but malignant (pathologic) lymphocytes with limited power of maturation, and their characteristics persisted after multiplication. Detailed gross and microscopic descriptions and numerous photographs were given.

Kirschbaum and associates have also contributed a number of studies. In one paper Kirschbaum and Strong described leukemia in the F strain of mice, and made observations on cytology, general morphology and transmission. Of the 17 cases, 13 were diagnosed as lymphatic leukemia and 4 as myelogenous. The distinctive morphology of each separate transmission line was emphasized. The morphologic features found in many leukemic cells were also present in normal cells and there was no specific cytomorphologic criterion for malignancy, although physiologically a great difference existed. The young cells of normal hemopoietic organs had certain cytologic characteristics in common with leukemic cells. Demonstrated by the dry-imprint technique, these were: *a*) nucleoli in the nucleus, *b*) a sievelike chromatin pattern, and *c*) cytoplasmic basophilia. The nuclei of the leukemic cells, however, often had more prominent nucleoli and were more hyperchromatic. Cells of the more differentiated leukemia were of a more mature type. No conclusive evidence was found that the introduction of leukemic cells into a normal susceptible mouse would provoke hyperplasia or metaplasia of host tissue, and the conclusion was reached that the resulting leukemia developed from the introduced cells.

Kirschbaum *et al.* (1940) compared transmissible lymphoid neoplasms which do not invade the blood with those which invade to a slight degree and those which invade readily. No cytologic differences in the cells

were recognized. It was concluded that the introduced cells, and not the hosts, determined whether the neoplasm would be a lymphosarcoma or leukemia. Work by Furth *et al.* (1935), however, has shown that exposure of the host to X rays, thereby lowering resistance, will affect the distribution of leukemic cells in myelogenous leukemia, and MacDowell *et al.* (1936) have shown that production of resistance by embryo tissue in the strain C58 host will modify a transplanted leukemia line, so that localized lymphosarcomas are produced in which the malignant cells have become "markedly different from those of the line inoculated." When transfers from these tumors were made to untreated strain C58 mice, characteristics of the transplanted leukemia line usually reappeared, although in a few instances the localized tumors continued. The lymphosarcomas were interpreted as "partially suppressed leukemia." Furth (1935), working with a myeloid leukemia, reported that localization or generalization was somewhat dependent on the route of injection, a generalized form developing after intravenous injection. Furth and Kahn in transmission studies with line S2, an atypical form, made successful transmissions in 5 mice out of 97 trials with a single cell only. Survival after inoculation was generally longer than when a large dose was given, indicating that the size of the dose affected the clinical course. Heilman and Kendall observed an inhibitory effect on lymphoid neoplasms in mice, produced by an adrenal cortical hormone. Regressions occurred, but the neoplasms later grew again, and were resistant to the hormone. The resistance appeared to reside in the cell, for when transplanted to new hosts, the resulting tumors were unaffected by the injections. Law (1951) made similar observations, and developed leukemic lines resistant to folic-acid antagonists. It thus appears that the characteristics of the disease can be modified both by changes in the host and in the cell.

The effect which genetic modifications may have on transmissible leukemia is uncertain. Divergent results in genetic studies may be due to differences in inbred strains. Schweitzer and Furth transplanted leukemias originating in the high-leukemia Ak stock to the low-leukemia Rf stock and to various hybrid combinations. They concluded that the duration of the illness and the anatomical characteristics were not modified by the genotype of the host. More recently Hogreffe (1948*b*) has performed a similar experiment using a high-leukemia Aka stock and a low-leukemia B stock. The transplants were made from spontaneous neoplasms developing in the Aka mice and not from long-established leukemic lines. On transplantation to pure line Aka mice, and to F_1 and ($F_1 \times$ Aka) backcross mice, a generalized leukemia developed in 100 percent of the cases. On transplantation to the F_2 generation, the percentage of successful transplants was reduced to 55 percent, and the resulting tumors were: 57 percent generalized leukemia, 22.7 percent localized growth, and the remaining intermediate. In the backcross to the B line, only 10 percent of the neoplasms grew, and the majority of these were localized growth.

(*3*) *Induced lymphocytic neoplasms.*—Three classes of external agents have been used to induce lymphocytic neoplasms. These are ionizing radiation, carcinogenic hydrocarbons, and estrogens.

The first successful induction of lymphocytic neoplasms resulted from irradiation (Krebs *et al.*).

The effects of irradiation on the reticular tissue of mice, and the protection against radiation death that may be accomplished by certain procedures, has already been discussed. It is not surprising that an agent producing such profound alterations should prove carcinogenic, and that modifications in tumor induction are elicited by altering the course of radiation damage. Many papers have appeared on this subject. Much of the work was summarized in a paper by Kaplan (1952*a*) in which he included many of his own observations carried out principally with strain C57BL.

The following points have been established by Kaplan and his associates:

1. Strain C57BL mice (Kaplan 1948*a*), which had a low spontaneous incidence, were highly susceptible to leukemia induced by irradiation but refractory to induction by other leukemogenic agents, such as methylcholanthrene and estrogens.

2. Susceptibility varied with the age at which the animal was subjected to radiation. It was maximal at 1 month of age or earlier (Kaplan, 1948*b*).

3. Dose and periodicity of irradiation caused a variation in incidence. Fractionated X irradiation at intervals of 4 to 8 days gave a higher incidence and shorter latent period than other intervals (Kaplan and Brown, 1952*a*).

4. A more recent paper (Kaplan, Nogareda, and Brown), which has already been discussed in regard to the effect of endocrines on normal tissue, also considered the relation of endocrine factors to lymphoid tumor development in irradiated mice (table 1). Orchidectomy and adrenalectomy, which caused hypertrophy of the thymus, also augmented lymphoid tumor incidence. Hypophysectomy had no effect. Testosterone injections greatly reduced the incidence of X-ray-induced leukemia in both intact and castrated strain C57BL males (Kaplan and Brown, 1951*a*). Cortisone inhibited the development of lymphomas in irradiated mice, while desoxycorticosterone had no effect (Kaplan *et al.*, 1951). Estrogens and ovariectomy failed to show a correlation in the effect on the thymus and in tumor induction, since large doses of estrogen caused involution, with later development of tumors in some strains and not in others, while ovariectomy caused hypertrophy of the thymus but no augmentation of lymphoid tumor development. The discrepancy cannot yet be explained.

5. Nearly all lymphoid tumors of this strain originated in the thymus and thymectomy effectively prevented the appearance of the disease after whole-body irradiation (Kaplan, 1950).

6. The neoplasms were lymphocytic or lymphoblastic lymphomas.

7. Local irradiation did not result in the development of lymphoid tumors with a frequency greater than the spontaneous incidence, whereas

irradiation applied over the whole body increased the number significantly (Kaplan and Brown, 1951*b*).

8. When parts of the body such as the thigh, lower extremity, or mediastinal region were shielded, thymic regeneration occurred more rapidly and there was a significant reduction in tumor incidence. Shielding the tail did not have this effect, indicating that the volume of active marrow in the tail was small (Kaplan and Brown, 1952*b*). A similar reduction in tumor incidence was produced by the injection of bone marrow following systemic irradiation (Kaplan *et al.*, 1953*a*).

In a later paper (Kaplan *et al.*, 1953*b*) it was shown that thymectomy after total-body irradiation suppressed subsequent lymphoid tumor development. Thymic lymphomas were found in many of the thymuses excised 10 to 12 weeks after exposure. Thymectomized mice implanted after irradiation with homologous thymic grafts had a significantly higher incidence of lymphoid tumors than mice receiving no implants. The tumor incidence was slightly lower if the thymus implant was itself irradiated. Kaplan and Brown (1954) implanted nonirradiated thymic grafts into irradiated thymectomized mice. Typical lymphoid tumors arose at the site of the transplant, an instance of the induction of a malignant tumor in a tissue not itself exposed to the carcinogenic agent responsible for the change. This experiment further illustrates the importance of the thymus in the development of lymphocytic neoplasms in some strains of mice.

Lymphoblastoma following the administration of carcinogenic tar was described by Brues and Marble. The neoplasm followed a more rapid course in the tarred mice, but was morphologically similar to spontaneous neoplasms. It was concluded that a carcinogenic agent might act as an extrinsic factor leading to the development of lymphoblastoma. The effect produced by methylcholanthrene on the latent period of "lymphomatosis" in dilute brown mice was described by Mider and Morton. The latent period was much shortened, and general lymphomatosis developed in 28 animals. Neoplastic cells were generally found in the lymph nodes, the spleen, and the liver, and when the liver was heavily infiltrated, anasarca was also present. Histologically, the architecture of the lymph nodes and spleen was obliterated by masses of atypical cells, which were larger than normal lymphocytes, with scanty cytoplasm and large, round, vesicular nuclei. Infiltration was found in many other organs. The bone marrow presented a striking picture, the marrow spaces being filled with atypical cells, which spread through the cortical bone into the vertebral canal and surrounding soft parts. The chief anatomical difference in the induced lesions was the preponderance of external lymphadenopathy as contrasted with internal lymphadenopathy in the spontaneous disease.

Leukemia was induced by both methylcholanthrene and by X rays in low-leukemia Rf mice, and in hybrids of Rf and high-leukemia Ak (McEndy *et al.*, 1942). The earliest microscopic evidence of leukemia consisted of large atypical cells frequently in mitosis, which were found in the splenic pulp and in reticulum meshes of the lymph nodes. The

neoplastic nature of these cells was proved by successful transmission to other mice, the biological test or bioassay. A number of the neoplasms produced were considered "atypical" and the origin from lymphocytes was not clearly established.

Rask-Nielsen has published a series of papers dealing with the effect of carcinogenic hydrocarbons on the development of tumors. Strain Street mice were used, in which stem-cell leukemia generally originating in the thymus occurred spontaneously. In one paper (Rask-Nielsen, 1948) a review of earlier investigations on the induction of tumors by the direct application of carcinogenic hydrocarbons was given. The hypothesis was advanced that carcinogenic action would only be capable of inducing the development of tumors in spontaneously tumor-producing tissue. Injections of 9,10-dimethyl-1,2-benzanthracene in 0.005, 0.02, and 0.5 mg. doses were made directly into the thymus, lymph nodes, spleen, bone marrow, lung, mammary gland, subcutaneous tissue, skin, testis, and kidney. The 0.005-mg. dose was ineffective, but when the higher doses were injected into the thymus a marked increase in the number of thymic tumors developed, and they appeared at an earlier age. Injections into the lung caused an increase in thymic tumors, and injection at other sites caused a relative increase in the ratio of thymic tumor to generalized leukemia and an increase in incidence and reduction in age in comparison with controls. It was pointed out in the discussion that the results supported the original hypothesis, and that the sensitivity of the thymus in comparison with the lymph nodes, spleen, and bone marrow might be explained by the active lymphocyte production in the thymus, and that in Street mice even generalized leukemia usually originated in the thymus. In a later paper (Rask-Nielsen, 1949) the same carcinogen in a 0.5-mg. dose was injected at various subcutaneous sites in male and female strain Street mice. A higher percentage of leukemia developed in the females and relatively more thymic tumors were found when injection was in the flank. The thymus (Rask-Nielsen, 1950*a*) was also found to be susceptible to the direct application of three other carcinogenic hydrocarbons: benzpyrene, dibenzanthracene, and methylcholanthrene. When the same four hydrocarbons were applied to the lungs (Rask-Nielsen, 1950*b*) an increase in thymic tumors resulted, as well as different types of tumors of the lung. When the hydrocarbons were given subcutaneously (Rask-Nielsen, 1950*c*) in 0.5-mg. doses, only 9,10-dimethyl-1,2-benzanthracene accelerated the spontaneous development of leukemia.

Removal of the thymus reduced the incidence of carcinogen-induced leukemia in strain DBA mice (Law and Miller). The carcinogen-induced leukemias did not differ morphologically from the spontaneous disease.

Leukemia induced by estrogenic hormones has been described by Lacassagne and by Gardner *et al.* No essential difference in morphology was found between cells from these neoplasms and spontaneous lymphocytic leukemia in the same strains of mice, although the distribution of the lesions might vary. The tumors reported by Gardner *et al.* often developed in the thymus.

Dmochowski and Horning reviewed reports on the induction of changes in lymphoid tissue by estrogenic hormones, and described an experiment of their own in which a local or general enlargement of lymph nodes, or thymus, or both, with or without invasion of the surrounding tissue, developed in castrated male mice painted with estrone. Histologically the normal cellular pattern of the lymph node was obscured by fairly uniform large lymphocytes. Mitoses were frequent, and similar cells invaded the capsule and penetrated the surrounding tissue. Transplantation was not successful, although histologically the changes resembled neoplasia.

The effects of leukemogenic agents in combination was investigated by Kirschbaum and Mixer. Using ionizing radiations, carcinogenic hydrocarbons, and estrogenic hormones, they induced leukemia in 8 inbred stocks which varied in susceptibility to the spontaneous disease. It was concluded that the genes determining susceptibility to the development of "spontaneous" leukemia were not identical with those involved in reactions to leukemogenic agents, and that a strain, such as CBA, with a relatively low incidence of spontaneous leukemia might respond to estrogen, but not to methylcholanthrene. Kirschbaum *et al.* (1953) tested the effect of three leukemogens—X rays, estrogenic hormones, and methylcholanthrene—on strains BALB/c, CBA, and DBA to determine whether there was a synergistic effect which would exceed the calculated additive expectancy. In BALB/c and CBA mice, X rays and estrogenic hormones acted synergistically, while methylcholanthrene was ineffective. In DBA mice, estrogenic hormone enhanced the leukemia-inciting potency of either the carcinogenic hydrocarbon or the ionizing radiations. The thymus was the most important site of development of lymphocytic neoplasms.

Gross (1951) claimed that an agent had been extracted from normal embryos, normal testes and ovaries, and leukemic cells of strain Ak mice (a high-leukemia strain), which when injected into newborn strain C3H mice (a low-leukemia strain) caused the development of leukemia many months later. After filtration of the extracts the neoplasms developed in 81 percent of inoculated mice at an average age of 10.6 months, and after centrifugation, in 46 percent at an average age of 5.2 months. This work was recently summarized and the biological properties of the agent discussed (1953*a*). No morphologic details have been described but it was said to be generalized leukemia indistinguishable from "spontaneous" leukemia frequently observed in Ak mice. It could be transplanted back into adult C3H mice (1951). Recently it was noted that many of the inoculated mice developed "neck" tumors, generally originating in the parotid gland, and morphologically different from any previously described tumors of this region in mice (1953*b*).

Dr. Lloyd Law has repeated phases of this work, and although the incidence of leukemia has not been notably increased as yet, parotid-gland tumors of unusual morphology, which I have examined histologically, have been found (publication in preparation).

A critical analysis of this work was given by Furth (1951, 1952). He pointed out that the experiments of Gross were vulnerable, because strain C3H mice are highly susceptible to grafts of Ak leukemic cells, and no simultaneous controls were carried. Filtration is difficult, and in every experiment of this type there must be proof that no cells were present.

(*4*) *Leukemia originating in the thymus.*—The importance of the thymus in leukemia induced by X rays and chemical carcinogens has been considered. The thymus is also significant in the development of spontaneous leukemia. McEndy *et al.* (1944) noted that the thymus was nearly always involved in the high-leukemia Ak stock of mice, and thymectomy considerably reduced the incidence while splenectomy had no such effect. Kaplan (1948*a*) made similar observations regarding X-ray-induced leukemia in strain C57BL. References to other studies of this type have recently been given by Law (1952) who reported an increase in the incidence of leukemia in hybrid mice that had received a thymic transplant from a high-leukemia parent strain. The resulting leukemia grew progressively on transplantation to the hybrid, but failed to grow when transplanted back to the parent strain from which the thymus was taken. This evidence supported the view that the lymphocytes which appear in the reconstituted thymus are derived from lymphocytes of the host. The surviving reticular tissue of the donor thymus apparently induced a malignant change in the infiltrating lymphocytes of the recipient.

Neoplasms originating in the thymus have not shown cytologic differences from lymphocytic leukemia originating at other sites in the lymphatic tissue, and behavior on transplantation was similar. Removal of the thymus did not affect the course of transplanted leukemia (Furth and Boon) or of other types of neoplasm.

Hogreffe (1948*a*) reported the spontaneous incidence of leukemia in the high-leukemia Aka stock and a low-leukemia B stock, and the first filial generation of crosses between these strains. He concluded that animals with "the strongest hereditary taint" died earlier, "often with enlargement of the thymus," in comparison with mice with a "less pronounced taint." The type of leukemia also seemed to be dependent on hereditary factors, since a greater morphologic variation was seen in the hybrids, and myelogenous leukemia was more frequent in older hybrid mice. The lines he established by transplantation from tumors originating in the thymus did not differ from lines derived by transplantation of tumors from other sites.

In a later paper in collaboration with Rask-Nielsen, the same groups of mice were injected subcutaneously with 0.5 mg. of 9,10-dimethyl-1,2-benzanthracene "in an endeavor to elucidate the interplay between the influence of genetic factors and carcinogenic agents on the development of neoplasms," particularly leukemia. A surprising uniformity in the incidence and types of leukemia resulted in the four groups of mice, despite the widely different incidence in the spontaneous tumors. The

accelerating effect of the carcinogen was noteworthy, since it was relatively slight in Aka mice with a high spontaneous incidence, and marked in B mice with a low spontaneous incidence. Thymic tumors were highest in Aka mice, lowest in B mice, and the hybrids were about midway between. In respect to the isolated thymic tumors, the carcinogen-treated mice corresponded to the untreated mice previously described by Hogreffe (1948*a*).

The thymus, therefore, is often a primary site for lymphocytic leukemia, particularly in young mice. The age incidence of this form of leukemia may be due to the greater importance of the thymus in young animals.

(*5*) *Cytologic studies.*—Cytologic studies on leukemia have extended the work on general morphology in attempting to answer these questions: 1) Is there a difference in the neoplastic and the normal lymphocyte? 2) Is there a morphologic change to accompany increased virulence? 3) Is there a morphologic difference in localized and generalized leukemia?

A series of cytologic studies were carried out on leukemia developing in strain C58 mice. Potter and Richter (1932) described the predominating cell type in transplantable line I. The cell resembled a large cell of the lymphatic series and its characteristics were large size (9 to 11 μ diameter), a moderate amount of cytoplasm containing many large round mitochondria and sometimes a few azure granules which were peroxidase-negative, and a nucleus usually irregular in shape, with many mitotic figures which were usually normal. The chromosomes numbered forty, the normal complement for the mouse. This cell was morphologically identical with cells found in embryonic lymphatic tissue. Later (1933) these authors described line I and two other transmissible lines and reported that the cell type for a given line remained constant for numerous transfers. In lines L and M spl B, the cells were between 6 and 8 μ in diameter and the shape was more nearly round than in line I. Azure granules were always present. Mitochondria were more abundant in line I and line L. The chromosome number was always forty. There was precocious splitting of chromosomes during division. Only slight differentiation occurred in the neoplastic cells, and there was no increase in the production of normal lymphoid cells by the host.

Potter and Ward (1942) compared the mitochondria in lymphocytes of normal mice and leukemic mice. Differences were demonstrated between the average number of mitochondria among populations of lymphocytes from the peripheral blood of normal mice, and mice with spontaneous or transplantable leukemia. More cells with a large number of mitochondria were found in the leukemic animals, indicating a different proportion of cell types. Large numbers of mitochondria were present in young blood-cell types. The same approximate stage of differentiation in the normal and leukemic cell had the same mitochondrial characteristics. It was, therefore, concluded that the pathologic cell was not distinguishable by an aberrant mitochondrial content. Miller and Taylor studied mitochondria in a transplanted lymphoid tumor exhibiting a change in virulence. Two of the same lines that were formerly used by Potter and Ward

were available. One of these, line I, had not changed in the interval, while line U had reduced its killing time from 15 to 4 days. A greater number of mitochondria was found in the normal lymphocytes than had previously been determined, but a proportionately greater increase was found in the line U leukemic cells. Line I remained as before. An increase in the number of mitochondria, therefore, appeared to be concomitant with an increase in virulence. Biesele studied chromosomes from the lymphocytes of normal C58 mice, mice with spontaneous leukemia, and mice inoculated with the highly virulent leukemia of long-transferred lines. He found a progressive increase in size, but there was no difference in microscopic complexity between chromosomes from the leukemic cell and chromosomes from the normal lymphocyte. When chromosomes of leukemic cells were partially digested by pepsin, they shrank more than the normal, suggesting that the greater size of chromosomes in the leukemic lymphocytes might be attributed to a greater amount of pepsin-digestible protein. Sex hormones had an effect (Biesele and Gasic) and it was demonstrated that testosterone propionate influenced the size of chromosomes, which were larger in male hosts and treated spayed females.

Shelton compared two transplanted lines of leukemia, one of which remained localized and killed in 30 to 40 days, while the other was quickly generalized and killed in 9 to 14 days. The cells of the localized neoplasm were approximately twice the size of the generalized leukemia and appeared to have a diploid number of chromosomes in the nucleus. No detectable alkaline phosphatase reaction occurred in the cells of the localized line, whereas the cells of the generalized line were heavily blackened. The localized tumor had relatively high acid phosphatase activity, while acid phosphatase activity in the generalized leukemia was low.

Hogreffe (1948*a*) also attempted to find morphologic differences in localized and generalized forms of leukemia. Nuclear size was measured, and in some cases the nuclei were found larger in the localized forms, which he interpreted as indicating a lesser degree of differentiation. Further work on this point was promised.

(*6*) *Behavior in tissue culture.*—Tissue culture has been tried as a means of distinguishing the leukemic from the normal cell, and cells of different types of leukemia from each other. Lewis (1938) noted in one line of lymphosarcoma that, "in appearance and locomotion the malignant lymphoid cells resembled greatly enlarged lymphocytes rather than lymphoblasts." Lewis and Mider also cultured 11 of the methylcholanthrene-induced "leukoses" described by Mider and Morton and 2 spontaneous "leukoses" in DBA mice. In their mode of locomotion, the cells from the induced neoplasms resembled myeloblasts from normal bone marrow, from leukemic blood, and from myeloblastic sarcomas. They differed, however, from lymphoblasts and mononuclear phagocytes obtained from normal tissue, and from cells obtained from lymphosarcomas and monocytomas. Characteristic cells from one of the spontaneous leukoses behaved like lymphocytes, while that of the other spontaneous

leukosis more nearly resembled large lymphocytes and the lymphoblasts found in normal lymph nodes and certain lymphosarcomas.

De Bruyn *et al.* have given a detailed description of the biologic behavior, the histopathology, and the results of tissue culture experiments on a lymphosarcoma line of the mouse. It was the authors' hope that a correlated study might determine which cells of the neoplasm were the vectors of the malignancy. The line had been carried for over 15 years. As in other leukemic lines, the interval between transplant generations became progressively less. No virus agent could be detected. The hemogram was followed closely after transplantation of the lymphosarcoma and a leukocytosis, partly due to an increase in monocytes, developed after 6 to 8 days, followed by a "shift to the left" in the granulocytes. In the final phase there were many tumor cells in the peripheral blood and a very high leukocyte count, with a relative lymphopenia. An extreme polychromasia developed before death. In microscopic sections the neoplastic cells were large, closely packed, round or polygonal, containing large nuclei and scanty cytoplasm. The nuclear membrane was conspicuous, the chromatin structure coarse, and one or two nucleoli were in evidence. Both nuclei and cytoplasm were strongly basophilic, and there were many mitoses. The liver and the spleen were the most severely involved organs after subcutaneous transplantation of the tumor; next in order were the lung, kidney, adrenal gland, and bone marrow. Only those lymph nodes draining the area of the subcutaneous tumor showed conspicuous growth, and the pancreas never contained tumor cells. The lumens of the larger veins in the lungs were often filled with tumor cells, and the bone marrow was almost completely replaced by tumor before death. The tumor cells appeared to be devoid of toxic effects and the mechanical displacement due to the massive involvement appeared to cause death. *In vitro* studies revealed 3 types of cells—lymphoblast-like cells, mesenchymal cells, and macrophages. By reinoculation of cultures into the mouse, it was proved that the lymphoblast-like cells were the carriers of malignancy, but in tissue culture these would grow only in the presence of the mesenchymal cells, and a kind of symbiosis appeared to exist. The morphologic and behavior characteristics of the malignant cells *in vitro* served to identify them accurately. The cells were larger than lymphocytes and contained a large nucleus with a few nucleoli. The chromatin structure of the nucleus was finer than the structure in a normal small lymphocyte. No peroxidase-positive granules were found. The cytoplasm was strongly basophilic and often contained small vacuoles, some of which could be stained with Sudan III. The cells showed no phagocytic activity. Their movement typically resembled that of lymphoblasts and lymphocytes. This is one of the most complete studies available, and it is regrettable that more debatable neoplasms are not subjected to such study, for some of them could then be identified with more assurance. In later studies de Bruyn (1949*b*) and de Bruyn and Gey developed from this line of lymphosarcoma a strain of cells containing no typical fibroblasts and growing without a supporting mesenchymal stroma. In early

subinoculations to the mouse these cells produced tumors, but later inoculations were unsuccessful. Even when mouse fibroblasts were added there were no successful subinoculations.

Bichel (1952) cultivated cells from a stem-cell leukemia, a chloroleukemia, and a plasma-cell leukemia of mice. A pure culture of leukemic mouse cells was obtained by the device of growing them in culture with chick-embryo fibroblasts, since the association with mesenchymal cells was necessary for growth. When transplanted back to the mouse, neoplasms having the features of the original lines developed. Bichel described the characteristics of the cells of the different lines in tissue culture, and noted the following features which distinguished them from normal, nonleukemic, hematopoietic tissue: 1) differentiation to more mature types was very limited, perhaps completely abolished, so that they did not mature beyond the stage at which they were able to multiply mitotically; 2) they never developed into other cell forms such as polyblasts, macrophages, or fibroblast-like cells; and 3) they could be maintained indefinitely in tissue culture as a characteristic cell strain. It was not possible to influence the maturation of the cells experimentally, and the hypothesis was proposed that the reason the leukemic cells are able to survive and multiply indefinitely in tissue cultures is that their potentiality for differentiation (maturation) is severely inhibited.

c) Characteristic case

The incidence of lymphocytic leukemia is usually highest in relatively young mice of high-leukemia inbred strains, in which it may affect 60 to 90 percent of all individuals before the age of 12 months. It may also appear sporadically in mice of "low-leukemia" strains, usually at a more advanced age. The incidence has been increased by irradiation, carcinogenic hydrocarbons, and estrogenic hormones, but different strains of mice do not react uniformly to the same agent and the incidence of induced leukemia may not parallel the spontaneous development. No morphologic difference has been detected between leukemic lines transplanted from induced or from spontaneous neoplasms.

Clinical symptoms may vary with different strains of mice. Palpable lymph nodes and spleen are usual in strain C58, and visible enlargement of cervical nodes in strain Akr. If the primary site is the thymus, as is often the case in young mice of certain inbred strains, difficult breathing may be the outstanding symptom. Examination of the peripheral blood may show a greatly elevated leukocyte count, with many abnormal cells in the blood smear.

At autopsy there may be general enlargement of the thymus (fig. 35), the lymph nodes, and the spleen (fig. 36). The involved organs are soft and white, and often show hemorrhagic areas. The surrounding tissue is usually edematous. Other organs or tissues may be involved to a variable degree; the kidneys, the ovaries, and the liver may be enlarged and pale, and contain nodules of soft white tissue. Ascites and anasarca are frequent in severe cases. Microscopically, a leukemic cell is found in the

blood smears (fig. 6), in organ smears, and in histologic sections (figs. 37–42). The leukemic cell may closely resemble a normal lymphocyte, and have a deeply basophilic small round nucleus, with a scant rim of clear basophilic cytoplasm (figs. 39, 41), or it may be much larger than the normal lymphocyte, and have a round or slightly indented vesicular nucleus which is less deeply basophilic, with one or more prominent nucleoli (figs. 40, 42). The cytoplasm may be clear, it may be distinctly basophilic, or it may contain a number of azurophilic granules. The cytoplasm is relatively abundant in the less differentiated types of lymphocytic leukemia. The mitochondria are generally larger and more abundant than in the normal lymphocyte, and the chromosomes are larger.

On transplantation of the neoplastic tissue from the primary growth in inbred animals of the same strain, a leukemic line may be established. Even when there is no gross evidence of the disease, and although no leukemic cells have been detected on microscopic examination, a neoplastic change may sometimes be demonstrated by transplantation trial to another mouse. This has been termed "the biologic test" or "bioassay" and is evidence that leukemia existed (Saxton *et al.*). A transplanted line displays distinctive biologic and morphologic characteristics. The killing time usually becomes shorter on successive transplantations, and although the greatest reduction occurs in the first few generations, some lessening of the interval may continue even after years of transplantation. Each leukemic line shows a characteristic behavior in the degree of localization or generalization, the organs that it involves, and the presence of leukemic cells in the peripheral blood. No difference after transplantation has been found in spontaneous or induced lines or in those originating in the thymus or from other sites. On microscopic examination, transplanted leukemia shows a more uniform cell structure than did the primary neoplasm, and a cell characteristic for the line may be recognized. This may closely resemble the normal lymphocyte, or may be much larger and resemble a lymphoblast. There are no constant morphologic features by which the leukemic cell can be differentiated from some stage in the development of the normal lymphocyte.

The characteristics of the neoplastic line are primarily dependent on the transplanted cell, but when transplanted to an irradiated host with lowered resistance, or to a partially immunized animal, or to a mouse of a slightly different genetic background, some variation in the degree of localization has been reported. The route of inoculation may also affect localization, a more generalized type developing after intravenous inoculation.

In tissue culture, cells from a lymphocytic leukemia have been larger than normal lymphocytes, but they have no absolute distinguishing feature. Movement is the same as that of the lymphocyte or lymphoblast. Only recently have the neoplastic cells been grown in pure culture, for mesenchymal cells are usually required in the culture along with the leukemic cells. They do not mature in tissue culture as do normal cells.

d) Differential diagnosis

Lymphocytic leukemia must be differentiated, first, from lymphoid hyperplasia and inflammatory infiltration by lymphocytes, and second, from other reticular neoplasms. Successful transplantation, the biologic test, is the most reliable method for determining the neoplastic nature of a lesion, but this is not always successful or practicable. Gross examination in which the diagnosis is made by noting the type and degree of involvement of the lymph nodes and other organs will give a high degree of accuracy in inbred high-leukemia strains, but microscopic confirmation is always desirable.

Lymphoid hyperplasia may often be recognized as secondary to an inflammatory process in the area drained by the enlarged node. Microscopically, the lesions already described under "Hyperplasia of particular cell types" and "Reactions to injury" may be seen. In these lesions, the normal architecture is usually maintained, and the capsule of the organ is usually intact. In a full-blown lymphocytic leukemia, the abnormal cells destroy the normal structure of the node, and invade and extend beyond the capsule. When small accumulations of a lymphocyte-type cell, somewhat larger than normal, are found in hyperplastic organs, it may be impossible to decide whether a lymphocytic neoplasm has developed at the site. It is our practice to diagnose leukemia only when indubitable evidence is present. Perivascular lymphocytic infiltrations are common in the lungs, the liver, the kidneys, the subcutaneous tissues, and at other sites in older mice, and it is not always possible to distinguish these from leukemic infiltrations. The cells in inflammatory reactions, however, are generally small with deeply stained nuclei, and are usually mixed with plasma cells and even a few cells with Russell's bodies. Extramedullary hematopoiesis and the leukemoid reaction offer no particular difficulties, since microscopic examination reveals the granulocytic nature of the cell.

Table 2 lists important criteria for differentiating the reticular neoplasms of mice. The criteria apply only to fully developed "typical" cases, and as discussed previously many gradations in morphology between neoplastic types are found. Lesions having a background of large reticulum cells with a heavy overlay of lymphocytic cells occur, and it is uncertain whether they should be considered as Type B reticulum-cell neoplasms (Hodgkin's-like lesions) with a preponderence of lymphocytic cells, or lymphocytic neoplasms. If the lymphocytic cells extend beyond the natural boundaries, and if they appear larger than the normal lymphocyte, then the diagnosis of lymphocytic neoplasm is made.

Distinction of a lymphocytic neoplasm from a well differentiated case of granulocytic leukemia offers no difficulty. When, however, the granulocytic leukemia is undifferentiated and of a "stem-cell" type similar to a lymphoblastic leukemia, differentiation may be impossible. Peroxidase-positive granules are not found in lymphocytic leukemia. Tissue culture is

TABLE 2.—*Differential diagnosis*

Neoplasm	Type of mouse	Effect of carcinogens	Clinical symptoms	Peripheral blood	Organs most often involved
(1) "Stem"-cell	Young mouse of high-leukemia Street strain.	Incidence increased and age decreased with chemical carcinogens	Palpable lymph nodes and spleen. Weakness, emaciation.	High white count—50 to 500 thousand or more per cu. mm. Leukemic cells nearly always present, at least terminally.	Lymph nodes, thymus, spleen, liver.
(2) Lymphocytic	Young mouse of high-leukemia strain.	Chemical, X ray, and estrogens increase, but with variations depending on strain.	Palpable lymph nodes and spleen. Difficult breathing when thymic. Weakness, emaciation.	High white count. Leukemia cells present in high proportion of cases. May be a leukemoid reaction also.	Lymph nodes, spleen, liver. Thymus often in young mice of certain strains.
(3) Granulocytic leukemia	Inbred strains with high incidence of lymphocytic leukemia. Small percentage of older mice of many inbred strains and hybrids.	No positive evidence	Palpable lymph nodes and spleen. Weakness, emaciation.	High white count. Leukemic cells with peroxidase-positive granules.	Spleen, bone marrow, lymph nodes.
(4) Reticulum-cell, Type A	Older mouse. In C57BL and sporadic in mice with low incidence of lymphocytic leukemia.	Incidence increased and age decreased with chemical carcinogens.	Enlarged liver and ascites. Enlarged uterus often.	Rarely neoplastic cells in peripheral blood, occasional monocytic leukemia.	Lymph nodes, liver, vagina and uterus. Occasionally spleen.
(5) Reticulum-cell, Type B	Old. Often in strain C57L mice.	No information.	Enlargement of mesenteric node in strain C57L. Later involvement of spleen and liver.	No report of abnormal cells.	Mesenteric node, liver, spleen, Peyer's patches, other internal nodes.
(6) Reticulum-cell, Type C	Strain C58 as a "preneoplastic" lesion. Rarely in other strains.	No information.	May be single palpable peripheral node.	No information.	Single lymph node, often cervical. Rarely liver and other nodes.
(7) Plasma-cell	Relatively common in old mice of Street strain, or C3Hf.	Apparently no effect.	Indistinguishable from lymphocytic leukemia.	Neoplastic plasma cells may appear.	Widespread in lymph nodes and other organs or localized development in ileocecal area in C3Hf.
(8) Mast-cell	Most often old hybrid with a strain A parent.	No information as to neoplasms. Hyperplasia after X ray, and chemical carcinogens.	No information.	One report of mast cells in peripheral blood.	Lymphatic tissue, and connective tissue.

Neoplasm	Macroscopic tissue	Characteristic cell	Histologic appearance	Transplantation	Tissue culture
(1) "Stem"-cell	White, hemorrhagic, soft.	Large, round "undifferentiated." No positive peroxidase granules.	Widespread infiltration with uniform leukemic cells.	Generally successful to same inbred strain.	Similar to lymphocytic.
(2) Lymphocytic	White, hemorrhagic, soft.	Large, round or may be smaller and resemble lymphocyte. Nucleus round, basophilic. Cytoplasm relatively scanty, basophilic.	Widespread infiltration with uniform leukemic cells. Occasionally larger cells clumped together.	Generally successful in inbred strains. Reduced killing time on passage. Individual lines have characteristic features.	Grown with mesenchymal cell. Characteristics of lymphocyte. Do not differentiate past certain stage.
(3) Granulocytic leukemia	Often a green color. Otherwise like lymphocytic.	Large cell with doughnut or bean-shaped nucleus. Peroxidase-positive granules.	Widespread infiltration leukemic cell showing cells in granulocyte series, with arrest at some stage.	Successful. Longer growth period than lymphocytic. Cells usually become less differentiated. Characteristic lines.	Described.
(4) Reticulum-cell, Type A	White, hemorrhagic, firm. Often flecks of orange color.	Medium size to large, and multinucleated often. May be abundant cytoplasm, vacuolated or containing ingested debris. Round or spindle shape.	Masses of tumor cells, usually no diffuse infiltration. Great variation in cell size and shape.	Successful. Very slow growth nearly always involving liver. Subcutaneous tumor may disappear.	Cells behave like monocytes.
(5) Reticulum-cell, Type B	White, soft.	Large, pale reticulum cell intermingled with a variety of other leukocytes.	Background of pale reticulum cells, overlaid with other leukocytes.	Rarely successful. Growth rate slow. Morphologic variation from original.	No information.
(6) Reticulum-cell, Type C	No information.	Large fusiform reticulum cell in masses, bordered by lymphocytes.	Sheets and cords of reticulum cells surrounded by small lymphocytes.	No information.	No information.
(7) Plasma-cell	Similar to lymphocytic neoplasm, but slightly firmer.	Various forms of plasma cells, from small typical to large, sometimes with several nuclei. Russell-body cell modification.	Resembles lymphocytic neoplasm except for type of cell. Sometimes areas with Russell-body-cell modification.	Rarely successful. Growth slow. Very few "takes" even in inbred strain.	One report of success.
(8) Mast-cell	Not distinguished from lymphocytic neoplasm.	Large mast cell, fewer metachromatic granules than normal.	Masses of mast cells, very little stroma.	No information.	No published report of success in mice.

helpful if facilities are available. Transplantation is of very little help since the same "stem-cell" type will emerge. A difficulty is also presented when a lymphocytic leukemia is accompanied by a granulocytic leukemoid reaction.

Distinction of lymphocytic neoplasm from reticulum-cell sarcoma is usually easy, except for the rare cases in which both forms of neoplasm are present in the same animal. Difficulty also arises in borderline cases in which the cells of the lymphocytic neoplasm are large, with a considerable amount of cytoplasm. Such cells may even appear oval or fusiform in shape and be clumped together, in contrast to the small, round, discrete and diffusely infiltrating lymphocytic cells of the typical lymphocytic leukemia.

e) Comparative pathology and discussion

Lymphocytic neoplasm is the most frequent form of reticular neoplasm in nearly all mammalian species. Engelbreth-Holm cited numerous case reports in a variety of mammals, and discussed the condition in dogs, swine, cattle, and rodents at greater length. Runnells and Benbrook described cases in the horse. Barile also observed a case in a horse and reviewed the literature. Single cases have been reported in the chinchilla by Newberne and Seibold, in an opossum and a Dorcas goat by Fox, and in the rabbit by Feldman. Medlar and Sasano cite other case reports in the rabbit. Among laboratory animals, Congdon and Lorenz described numerous personally observed cases in guinea pigs and reviewed the literature; Shay *et al.* cited reports in the rat, and described cases of their own; Eyestone cited cases diagnosed as lymphocytic leukemia in the cat; and Bloom and Meyer observed 20 cases in dogs and cited many preceding reports. They called attention to the frequency with which widespread extramedullary myelopoiesis had been erroneously reported as myelogenous leukemia in the dog, when actually, "no unquestionable cases of this disease have been described in dogs." Mulligan also described lymphocytic neoplasms in dogs. Steiner and Bengston noted that it was the commonest type of tumor listed on post-mortem inspection by the Bureau of Animal Industry in the United States in 1949, and that the figures were probably conservative. Both lymphoblastic and reticulum-cell types were included in the report. Cases were reported in sheep, cattle, and swine.

The condition in cattle is of especial interest since it appeared to be endemic in certain districts of East Prussia. It sometimes affects young calves. In a recent study Fortner has compared the disease in cattle with leukosis in mice, and concluded that both are on an hereditary basis.

Lymphocytic neoplasms are the most common form in the mouse and are found in a great variety of inbred strains. Slye, and Engelbreth-Holm have stated that these neoplasms are not found in wild mice. Gross examination of wild mice kept in Dr. Andervont's laboratory at the National Cancer Institute showed a number in which there was enlargement of the thymus and lymph nodes. Only two of these were suitable

for microscopic study, and the histologic picture was complex, resembling a reticulum-cell rather than a lymphocytic response. It is hoped that a detailed report of these lesions can be made if more cases become available.

This is the form which has most often figured in experimental work and most of the generalizations concerning experimental leukemia have been arrived at from studies of the lymphocytic neoplasm. Morphologically, it probably has a closer resemblance to the corresponding neoplasm of man than any other neoplasm found in the mouse, and microscopic fields from neoplasms in the two species may be indistinguishable (Furth *et al.*, 1935). It has a number of similarities to acute lymphocytic leukemia in children, including its clinical course and response to chemotherapy. Any new knowledge that we gain of this disease in mice may be directly applicable to man.

3) Granulocytic Neoplasms and Extramedullary Hematopoiesis

The diagnosis of granulocytic leukemia in the mouse is especially difficult because of the histologic similarity to extramedullary hematopoiesis and leukemoid reactions which frequently occur. An awareness of the frequency and extent of blood-formation at sites outside the bone marrow and the extreme leukocytosis which may develop in the peripheral blood is important in order not to confuse this reaction with granulocytic leukemia. The two conditions are therefore compared and discussed together.

Extramedullary hematopoiesis

a) Terminology.—This is often referred to in mice as nonmalignant extramedullary hematopoiesis, but with our present understanding of the condition, nonmalignant is an unnecessary specification. When the outstanding activity is in the granulocyte series, granulocytopoiesis may be specified; if nucleated red cells are the predominant cell, erythrocytopoiesis is an appropriate term. Myeloid metaplasia is a synonym commonly used. Leukemoid reaction is the term applied to a greatly elevated leukocyte count in the peripheral blood.

b) Previous descriptions.—Barnes and Sisman gave a detailed description and tabulated the important points for differentiating extramedullary hematopoiesis from granulocytic leukemia (table 3). It is probable that many of the early reports on leukemia in the mouse failed to recognize the non-neoplastic condition, extramedullary hematopoiesis, so that many early descriptions of "myelogenous" leukemia cannot be accepted without reservation. Thus, Hill described a "mixed leukemia" which varied from a lymphoid to a myeloid type. Yet experimental inoculation of a lymphocytic leukemia has never resulted in a granulocytic leukemia, nor has the reverse been shown. Simonds (1925*b*) found 29 cases of myeloid and 28 cases of lymphatic leukemia in the Slye stock of mice, but it is not certain that he made a distinction between myeloid leukemia and a leukemoid reaction. It is also problematic whether Andersen clearly appreciated

leukemoid reactions, for he described forms of leukemia composed of cells with abundant rich cytoplasm and ring-shaped or lobed nuclei in which megakaryocytes were also increased. Parsons (1935) described a fibrosarcoma with an accompanying "leukemia," which was later considered a leukemoid reaction (1936), and others have reported a similar increase in granulocytes accompanying tumor growth (Lewis 1937). Rask-Nielsen reported that "a transmissible myeloid leukosis" produced by irradiation (Krebs *et al.*) was accompanied by a pronounced neutrophilia. Attempts to find a responsible agent in the leukotic cells or a secondary bacterial infection which produced this effect were unsuccessful.

TABLE 3*

Myeloid leukemia	Nonmalignant extramedullary myelopoiesis
Most myeloid cells are immature	All stages in the development of myeloid cells are present.
Erythrogenic foci are absent among myeloid cells	Erythrogenic foci are usually present.
Megakaryocytes are few and present only in the organs (spleen, liver, and lymph nodes) where they are found in nonleukemic conditions	Megakaryocytes are usually numerous.
Myeloid cells often invade muscle and other nonhematopoietic tissues	Cells are noninvasive.
Blood usually contains immature myeloid cells	Blood is either normal or there is leukocytosis with numerous mature forms.
Liver is usually enlarged and gray-brown	Liver is usually not enlarged and is brown-red.
Most of the lymph nodes are usually enlarged	Most of the lymph nodes are usually of normal size.
Hemorrhages are frequent in viscera (lungs, lymph nodes, etc.)	Hemorrhagic manifestations are absent.
Transmissible to other mice	Not yet shown to be transmissible.
Not shown to be produced by bacteria	Can be produced by bacteria.

*After Barnes and Sisman.

Bateman, in this laboratory, described the granulocytic increase resulting from the growth of three transplantable myoepitheliomas and a fibrosarcoma; a reduction in granulocytes followed surgical removal of the tumors. The paper by Bateman gave references to preceding reports.

Weir and Mahler recently reported an "apparent chronic myeloid leukemia practically indistinguishable" from the human disease, in Swiss mice following intermittent cortisone therapy. No transplantation attempts were reported, and this work will need to be confirmed and ex-

tended before it can be accepted as a granulocytic neoplasm rather than a leukemoid reaction and extramedullary hematopoiesis.

c) Characteristics of extramedullary hematopoiesis.—A moderate degree of extramedullary hematopoiesis is physiologic in the spleen of the mouse, and is always present in normal young animals. Growth of certain neoplasms may act as a stimulus to a more pronounced reaction. Inflammatory conditions such as localized abscesses, acute pyelonephritis, and pneumonia may be found at autopsy in mice having an increased number of granulocytes. A recent report by Widmann described the production of a leukemoid reaction by the injection of colchicine, but we have not been able to confirm this. In some cases no associated disease can be found to account for the extramedullary hematopoiesis and the greatly increased leukocyte count in the peripheral blood. Repeated blood loss, hemolytic anemia, or neoplastic growth may cause an increase in erythrocytopoiesis, but histologically this is easily distinguished from granulocytic leukemia. When there is an extreme demand for erythrocytes or granulocytes or both, the spleen is usually enlarged; the liver may also be enlarged and exhibit small white nodules, and even the lymph nodes are enlarged in severe cases. Histologically immature forms of erythrocytes and granulocytes are found in the red pulp of the spleen, the preponderance of one or another cell type usually depending upon the stimulus. In extreme cases the adrenal gland (fig. 43), the ovary, the peripelvic fat tissue of the kidney, and even the pituitary gland may show blood-cell formation. Extreme degrees of leukocytosis up to several hundred thousand cells per cu. mm. may be found in the peripheral blood. The granulocytes appear to develop most actively beneath the capsule of the spleen and along the trabeculae, where large immature mononucleated cells can be seen, while more mature cells with ring-form nuclei are found within the sinusoids. An increase of megakaryocytes usually accompanies an increase in granulocytes. The splenic nodules take no part in the reaction. In the liver, the granulocytic activity seems greatest in the tissue adjacent to the portal vein. The walls of the vein may be heavily infiltrated, so that the layers of the venous wall are separated by the cells. Small clumps of immature white cells, megakaryocytes and nucleated red cells may also be found within the sinusoids of the liver if the reaction is extreme. In the lymph node, hematopoiesis is active in the medulla, the cortex being little affected. In the adrenal gland, the foci are generally found in the cortex (fig. 43). In the pituitary gland the immature blood cells have been found among the cells of the anterior lobe. In severe leukemoid reactions the lungs contain numerous granulocytes in the larger vessels and alveolar capillaries (fig. 44).

d) Differential diagnosis.—The most important criteria for differentiation from granulocytic leukemia are given in table 3. No other lesion presents any difficulty.

e) Comparative pathology and discussion.—Extramedullary hematopoiesis and leukemoid reactions are described in many laboratory animals. Engelbreth-Holm cited references to rabbits, monkeys, and dogs, and

Bloom and Meyer emphasized the frequency with which it is erroneously diagnosed as leukemia in dogs. Kelsall (1949) described hematopoiesis in the spleen of hamsters. Miller and Turner observed changes which were probably of this nature in guinea pigs following the injection of substances obtained from the urine of patients with myeloid leukemia. The lesions in guinea pigs were thought to be similar to human chronic myeloid leukemia, and extracts from the urine of patients with chronic lymphoid leukemia were said to reproduce human chronic lymphoid leukemia. Lang reviewed much of the experimental work in guinea pigs.

Forkner, who referred to the condition as "leukemoid states (secondary or symptomatic leukemia)," cited many diseases in man accompanied by a leukemoid reaction. Under the term "aleukemic myelosis," Heller *et al.* listed numerous terms that have been used in the past, described three cases of their own, and cited many preceding reports. It was their opinion that many of the cases so diagnosed in man probably represented myelogenous leukemia. Reports of leukemoid reactions accompanying neoplastic growth in man are infrequent in comparison with reports in mice. Hinshaw and Hoxie described a leukemoid reaction associated with carcinoma of the bronchus, and cited references to others.

Leukemoid reactions in animals should be sharply differentiated from true leukemia, since the biological nature of the two processes is distinct. A severe leukemoid reaction, even when long continued, has never been proved to induce leukemia, and transplantation attempts have failed. However, the difficulty of differentiating the non-neoplastic from the neoplastic production of granulocytes by morphologic criteria alone has caused much confusion in the past, for it is often impossible to differentiate the two conditions in a given case.

Granulocytic leukemia

a) *Terminology.*—Many terms are used for this neoplasm, but in accordance with the recommended terminology, "granulocytic leukemia" is adopted. Other names used for the neoplasm in mice are: myelosis, myelogenous leukemia, myelocytic leukemia, myeloblastic leukemia, and myeloma (if the neoplasm is localized). Chloroleukemia has been used when a green color developed in the neoplasm.

b) *Previous descriptions.*—Granulocytic leukemia has usually arisen in stocks of mice in which lymphocytic leukemia is frequent. Consequently descriptions of this type of leukemia are usually found in reports on the more frequent type. Barnes and Sisman published a comprehensive paper, in which particular consideration was given to the differentiation between extramedullary hematopoiesis and granulocytic leukemia. They reviewed earlier reports of granulocytic leukemia, including papers by Simonds (1925*b*), Furth (1935), Hall and Knocke, and Kaalund-Jørgensen (1935).

Furth (1935) described the first successful transplantation of myeloid leukemia in mice. A myelocytic cell with coarse basophil granules was characteristic of the transplanted line. The spleen, the liver, the bone

marrow, and the lymph nodes contained many of the neoplastic cells. Transplantation was more successful in irradiated mice. It was determined that as few as an estimated 11 cells could result in successful transplantation. When intravenous inoculation of a relatively large number of cells was employed, a systemic diffuse disease (myeloid leukemia) and death in 2 to 3 weeks resulted. Subcutaneous or intraperitoneal inoculation, or resistance in the inoculated mice, resulted in localized tumors composed of the malignant blood cells, and the course might be chronic lasting 2 to 5 months. From these observations it appeared that localization or generalization might vary depending upon the resistance of the host and the route of transplantation. The characteristic cell differed morphologically from a nonmalignant, immature, myeloid cell. The granules did not give the oxidase reaction.

Kaalund-Jørgensen (1936) carried out numerous transmission experiments on a transmissible line developed by Krebs *et al.* He concluded that it was "of a myeloid nature, and intimately connected with the reticulum." The "true tumor cells" were of various shapes—polygonal, not round—and the limits of the cytoplasm were ill-defined. The cytoplasm was basophilic, sometimes vacuolated, and sometimes contained granules. The nucleus was rounded, but might show one or more indentations, and there was a concentration of chromatin at nodes of the nuclear network, usually with one large chromatin granule lying almost in the center, and several smaller ones distributed along the nuclear membrane. Cells in the peripheral blood were peroxidase-negative. In comparison with Furth's myelosis (1935), the cells were very immature.

The description and photographs resemble cases that I have classified with lymphocytic leukemia, in which the cells are much larger than the normal lymphocyte, and have a round or slightly indented vesicular nucleus with one or more prominent nucleoli (figs. 40, 42). This type is less differentiated, and there is always uncertainty as to its exact position.

Rask-Nielsen and Rask-Nielsen studied this same strain, and noted its behavior both in nonirradiated mice to which it was successfully transplanted, and in irradiated mice. A marked neutrophil leukocytosis, with an "autochthonous origin of pathological myeloblasts in the marrow" appeared in the inoculated mice. Details of the changes in the peripheral blood in both groups of mice were given as well as the gross and microscopic appearance of the tissues. Irradiation did not seem to alter the development of the anatomical changes in organs due to propagation of the inoculated cells, whereas the reaction in the peripheral blood and bone marrow was "altered or veiled" by the irradiation, probably because of injury to the marrow and failure of leukocyte reaction. A resemblance to the "leukosis" accompanying growth of a transmissible spindle-cell sarcoma described by Parsons (1935, 1936) was pointed out. This appears to be another instance in which a leukemoid reaction accompanied the growth of a neoplasm, and complicated the diagnosis.

Storti also studied myeloid leukemia in the mouse. He obtained the leukemic line isolated by Furth (1935) and carried in the Rfd strain.

Storti was interested in determining whether myeloid leukemia was truly neoplastic, and whether acute and chronic forms were etiologically identical. When the leukemic cells were injected subcutaneously, a localized tumor developed, and blood-stream invasion occurred at varying intervals thereafter. On intravenous injection the cells initially disappeared from the blood stream, but were rapidly established in the bone marrow, and were later disseminated to the blood stream. Considerable variation was found in the course of the leukemia, depending upon the route of injection and even more upon the condition of the injected animal. Storti believed that the conditions of acute or chronic leukemia similar to those observed in man were thus reproduced in the mouse, and he considered the course of the disease in mice proof that acute and chronic leukemia are basically the same, although anatomic and clinical differences might be manifested. He also concluded that autochthonous foci did not develop, and that all leukemic areas arose from the introduced cells, which were truly neoplastic.

Hall and Knocke described a transmissible chloroleukemia. Similar green-colored neoplasms have been infrequently observed in mice by others. In the animal with chloroleukemia the spleen and lymph nodes were enlarged, and the latter were green in color. Transplantation was successful, and with continued transplantation the killing time decreased from 26 days to 16 days. The spleen in the mice receiving the transplant was greatly enlarged, and there was enlargement of all or most of the lymph nodes. On microscopic examination, there were two types of cell: 1) large cells with very little cytoplasm and large, irregular, rounded, hyperchromatic or vesicular nuclei having large acidophilic nucleoli, or 2) large cells with a relatively large amount of cytoplasm and bean-shaped nuclei. Mitotic figures were numerous. The lymph nodes, liver, lungs, kidneys, and bone marrow were the organs most often involved.

Barnes and Sisman found myeloid leukemia frequent in two stocks of their mice. In one of these, Rf, lymphoid leukemia was unusual, while the other, S, exhibited both lymphoid and myeloid types and atypical forms. The morphologic characters were given by which myeloid leukemia was diagnosed, and the morphology of different stages in the development of the normal granulocyte was described.

Kirschbaum and Strong described four cases of myelogenous leukemia and thirteen other spontaneous cases of lymphatic leukemia or lymphosarcoma in the F strain of mice. Three of the four cases of myelogenous leukemia appeared to be chronic, and two had greenish lymph nodes. (In this same paper greenish lymph nodes were also described in a case of lymphatic leukemia.) One line became less differentiated after transfer, but peroxidase-positive cells were still found. This same line contained a notable number of mast cells. It was remarked that in one line a number of leukocytes in the neoplastic tissue and peripheral blood were probably not of leukemic-cell parentage. The authors stated that the stem cells of lymphatic and myelogenous leukemia might be quite similar, although so-called myeloid azure granules were seldom seen in lympho-

blasts while they were not infrequent in leukemic myeloblasts. In an addendum to this paper the authors reported that more cases of leukemia were subsequently observed in this strain. The proportion of myelogenous leukemia continued to be about one-fourth of the total, and the remaining three-fourths were lymphatic.

Menkin injected mice of the Ak strain, inoculated with chloroleukemia, with his leukocytosis-promoting factor and observed a shift in the differential leukocytic formula. The percentage of mature polymorphonuclear leukocytes increased in the circulating blood.

c) Characteristic case.—Granulocytic leukemia has been observed rarely in mice at the National Cancer Institute. It has most often appeared sporadically in old hybrid mice in which the incidence of other forms of leukemia was high. Reports from other laboratories indicate that granulocytic leukemia may be especially frequent in some stocks of mice (the Rf reported by Barnes and Sisman), but it has not been found preponderantly in any one inbred strain. Therefore, there has been no opportunity for a controlled study of granulocytic leukemia such as has been made with lymphocytic leukemia in strain C58 mice. Clinical symptoms are indistinguishable from lymphocytic leukemia. A high leukocyte count with many undifferentiated cells of the granulocytic series is found in the peripheral blood. At autopsy, a distinctive green color may sometimes be seen in the neoplastic tissue. Microscopically, extramedullary hematopoiesis presents an obstacle to exact identification. Transplantation has been successful, but the resulting line may be more undifferentiated than the primary neoplasm and fail to reveal the characters required for an exact diagnosis. Reports indicate that many transplanted lines do not acquire such an increased "virulence" as do lines of lymphocytic leukemia.

d) Differential diagnosis.—Criteria for differentiation from extramedullary hematopoiesis are given in table 3, and from other neoplasms in table 2. Localized inflammatory processes with a preponderance of granulocytes are distinguished by the maturity of the cells and the focal nature of the reaction. When only microscopic sections are available, collections of relatively immature granulocytic cells, most of which appear to be in the same abortive stage of differentiation, are indications of granulocytic leukemia. If the cells are located in areas such as the peripelvic fat tissue of the kidney in which extramedullary granulocytopoiesis is seldom found, they have more significance in indicating leukemia. Infiltration in the kidney is sometimes found (fig. 45). Extension beyond the capsule of a lymph node is also suggestive of neoplasm. The Giemsa stain is often helpful since it shows more clearly the morphology of leukocytes.

The differentiation of granulocytic leukemia from stem-cell leukemia depends upon a positive peroxidase reaction in the granules.

Separation from lymphocytic leukemia should offer no difficulty provided that the neoplastic cells are sufficiently differentiated. Lymphocytic leukemia may be accompanied by a leukemoid reaction which com-

plicates the problem of diagnosis, and this possibility should always be kept in mind.

The rare chloroleukemia is recognized by the green color seen in the fresh tissue. This color is especially prominent at the ends of the long bones. It can be suspected in histologic sections when many large mononuclear cells with eosinophilic or brown-tinted cytoplasm are found intermingled with the neoplastic granulocytes (fig. 46).

The problem of differentiation from the reticulum-cell neoplasm is rarely presented, unless the diseases are coexistent, since morphologically there is little resemblance.

e) Comparative pathology and discussion.—In comparison with lymphocytic leukemia, granulocytic leukemia is rare in lower mammals. References to scattered case reports in a number of species are given by Engelbreth-Holm. It has been reported a number of times in rats, and in comparison with other forms of leukemia it is probably relatively more frequent in rats than in mice. Wilens and Sproul described a number of cases of chloroleukemia, and several cases in rats have been seen in this laboratory. A large mononuclear cell with abundant cytoplasm is frequently found associated with the leukemic cells. With hematoxylin and eosin the cytoplasm of the mononuclear cell may be intensely eosinophilic or diffusely brown in color. This cell appears to be characteristic of chloroleukemia, but whether it has any relation to the intense green color of the tumor has not been determined. Shay *et al.* found granulocytic leukemia in Wistar rats following gastric instillation of methylcholanthrene. They cited important previous reports.

Eyestone described myelogenous leukemia in a cat, and cited references to preceding reports.

The relative rarity of granulocytic leukemia in the mouse in comparison with the relatively high incidence of this disease in man is puzzling. Several species differences may be significant. Lymphocytes in the peripheral blood of the mouse normally outnumber the granulocytes by a ratio of 3 or 4 to 1, while the reverse is true in man. The lymphatic organs in the mouse remain relatively active throughout life, whereas in man a greater degree of atrophy occurs in old age. Histologically, some cases in the mouse have a close similarity to human cases, and the relatively chronic course is clinically similar.

4) Reticulum Cell

The solitary fixed cells forming the cellular elements of the reticular framework of lymphatic and hematopoietic organs give rise to several distinctive neoplastic diseases in the mouse. Two of these, Types A and B, are proved to be neoplastic, at least under certain conditions, and the third, Type C, is doubtfully so. Other less distinctive neoplastic alterations are found in the reticulum cells, and there are many borderline cases between a hyperplastic and a neoplastic lesion and between different neoplastic lesions. Such complications make neoplasms of reticulum-cell origin especially difficult to characterize and classify. Information regard-

ing these lesions is limited due to their relative infrequency, especially in young mice.

In previous publications from this laboratory, the lesion now termed Type A was called reticulum-cell sarcoma, and Type B was termed the Hodgkin's-like lesion after the closest human counterparts. This often led to arguments regarding the similarity or difference of these neoplasms in the two species, and controversy as to the appropriateness of the terms. To avoid this entanglement in semantics it was decided to adopt non-specific terms having no particular connotation, and reticulum-cell neoplasm Types A, B, and C have been adopted.

Reticulum-cell neoplasm, Type A (reticulum-cell sarcoma—localized, and monocytic leukemia—generalized)

a) *Terminology.*—Previously used terms are: transferable liver neoplasm, polymorphous-celled-sarcoma, reticulo-endotheliosis, reticulo-endothelioma, monocytoma, histiocytoma, monocytomatosis, and *leucemie reticulaire* (French). "Monocytic leukemia" has been used when the neoplastic cells were found in the peripheral blood.

Furth (1939) thought it desirable that the term "reticulum-cell sarcoma" be dropped, and these tumors be referred to as histiocytoma or histiocytomatosis, or monocytoma or monocytosis, since they differ in many respects from neoplasms in man. There is much to support this point of view, but since the neoplastic cell in the mouse originates from the reticulum cell and displays many characteristics of that cell, the term reticulum-cell neoplasm, Type A, is appropriate. When the neoplasm is localized, it is referred to as reticulum-cell sarcoma, and when generalized as reticulum-cell neoplasm—generalized, or as monocytic leukemia.

b) *Previous descriptions.*—Descriptions of this tumor have appeared with extraordinary frequency. Although Furth and Furth described "a leukemia-like disturbance with malignant cells resembling monocytes" in 1938, as a disease "hitherto not described in mice," several previous observers had described conditions which probably belong in this category. Haaland (1911) examined a mouse 24 months of age and described a "polymorphous-celled-sarcomatous tumor consisting of spindle-shaped and round cells mingled here and there with larger elements and even giant cells." It was successfully carried through only one passage.

Tyzzer in a study of a series of spontaneous tumors listed three "unclassified" tumors, two of which were suspected of being of endothelial-cell origin because of the proliferation of cells within the endothelial lining of veins. These tumors were similar in all respects and the animals bearing them had enlarged livers. Descriptions and photographs leave no doubt that this is the same neoplasm which we have called reticulum-cell sarcoma, Type A. Among spontaneous primary tumors involving the liver, six lesions composed of spindle cells and having similar cells in the spleen and lymph nodes were described by Slye *et al.* In four cases there was plugging of some of the vessels in the liver with masses of cells of "a more endothelial type." The authors concluded, however, that this process was an inflammatory granuloma and not neoplastic.

Woglom described a "carcinoma of the uterus in a mouse" that was similar to reticulum-cell neoplasms, Type A, which we have often seen in female mice. The frequency with which this neoplasm originates in the wall of the uterus or vagina is notable.

Lignac injected mice repeatedly with benzol and observed in three a lesion that affected the liver in all and the spleen in two. Two mice died with ascites, and one female had an enlargement of the uterus. Microscopically the liver and the uterus showed a neoplastic growth of hematopoietic tissue, which was locally connected with the endothelium of the blood vessels. Lignac remarked that these three cases were a striking analogy to human cases of monocytic leukemia. He observed that in the liver the endothelial cells adjacent to the central vein might become rounded and discrete, and show erythrophagia. The author considered that the endothelial origin of monocytes was clearly demonstrated by these preparations, and he reviewed a number of papers in human pathology upholding this opinion. He also discussed reports of "reticulo-endotheliosis" in man, which had a resemblance to the condition in the three mice described by him. Heim and Schwartz noted in an appendix to a review of Simonds' paper (1925*b*) that both Slye's and Tyzzer's papers contained cases similar to those described in human beings as "reticulosis," in which there occurred a benign systemic growth of elements of the reticuloendothelial apparatus with striking enlargement of the spleen, liver, and lymph nodes.

Andersen also described and pictured, among his "special forms," several cases of "reticulo-endotheliose," and "polymorphous cell sarcoma," which in all probability were examples of this tumor type.

Furth and Furth in 1938, however, clearly differentiated this neoplasm. The tumors resulted from an intrasplenic injection of 1,2-benzpyrene and were found in eight mice of the Rf stock. The condition was referred to as monocytic leukemia or monocytomatosis. It was not found in untreated mice of this series, although it had been found in Rf stock mice a few times before. The eight affected mice were three males and five females and ranged in age from 7½ to 16 months; six of them were over 12 months of age. The pathologic process was characterized by an overgrowth of mononuclear cells, mainly in the liver, and to a lesser extent in the spleen and lymph nodes. Invasion of the vessels of the liver developed. A resemblance to Hodgkin's disease was noted in some cases. Other features mentioned were occasional giant cells, and the phagocytosis of red cells by monocytes. One case showed involvement of the ovary and uterus and in one case the neoplastic process appeared more diffuse without local tumor formation.

Cloudman (1939) described a "transferable liver neoplasm" in strain C57 leaden. In the original tumor the liver and spleen were involved. On subcutaneous transplants, small local growths developed which disappeared, to be followed by liver enlargement and ascites. Microscopically, spindle cells which appeared to originate from the endothelium were found in the hepatic sinusoids. Tissue that was grossly negative

for tumor was taken from a variety of sites, and on transplantation growths resulted. Hybrids of strain C57 leaden had only a few successful transplants, whereas in pure-strain mice of the parent strain the tumor was successfully transplanted in 100 percent of the cases. This was an unexpected result, since most tumors take equally well in hybrids in which one parent is of the same inbred strain as the mouse bearing the primary tumor. A tentative diagnosis of "reticulo-endothelioma" was made.

In 1947, Cloudman again discussed this tumor. He reported that the tumor originating in 1936 had been carried for more that ten years by transfers from the liver to subcutaneous areas. The subcutaneous growths were negligible, and often too small for transfer. When the tumor was transplanted to other pure strains no growth resulted, except when C57BL mice were in parabiosis with C57 leaden, the strain in which the tumor originated. Under these conditions about 90 percent grew the tumor, which was then successfully transplanted to intact C57BL mice. No histologic difference in the tumor was found between the two strains. When the tumor was transplanted subcutaneously to susceptible hybrid mice, a difference in distribution of the internal lesions was noted, and the author concluded that these diverse organophilic tendencies were due to differences in the host.

Mider and Morton described a "reticulo-endotheliosis" in one of sixty dilute brown mice painted with methylcholanthrene. Phagocytosis of erythrocytes and involvement of the portal areas of the liver were noted.

Lewis (1939) described a "transmissible monocytoma" in a mouse of the C57 strain which had been injected 411 days previously with dibenzanthracene. A small spot on the spleen proved to be a "monocytoma" which grew slowly after subcutaneous transplant. Metastases occurred first to the liver, and later to the spleen, thymus, and bronchial lymph nodes. Tumors developed after transplantation of grossly normal tissue. Growth rate of the tumor varied. Lewis described the lesions as having the "appearance of a tubercle whose cells have become cancerous." Epithelioid cells, multinucleated giant cells, macrophages, monocytic cells resembling Sternberg cells, and multinucleate Langhans-type giant cells were described. The tumor cells were phagocytic and contained ingested erythrocytes, cell debris, and bilirubin crystals. Some cells were elongated and spindle shaped. The tumor was later grown in tissue culture, and here the phagocytic property of the cells was also exhibited. The tumor was compared to Furth's monocytic leukemia in tissue culture, and while differences were noted, it was concluded that the cells from which these tumors were derived belonged to the same group.

Furth in 1939 further described leukemia with malignant cells related to histiocytes. The mouse was of the Rfb stock, 24 months of age, and the tumor was spontaneous, involving spleen, liver, and lymph nodes. Reports on seven transplant passages were given. Transfer was successful to hybrid mice obtained by crossing the susceptible parental stock and another stock. Either a small local growth or no detectable growth resulted, but the liver became enormously enlarged. Microscopically the

infiltration of the liver was diffuse, with the formation of nodular tumor masses. Malignant cells often invaded vessels of medium size, almost completely occluding them. It was noted that the malignant cell assumed many different forms, and giant cells like those seen in Hodgkin's disease were found in large numbers in occasional mice. What appeared to be malignant monocytes were found in the peripheral blood and sometimes constituted 23 percent of the differential count. Cells from this tumor line were grown in tissue culture, and the behavior was described.

Little *et al.* noted the number of reticuloendotheliomas of the liver appearing in strain C57BL and strain DBA mice and in their hybrids. No morphologic descriptions were given. The tumors were especially frequent in C57BL mice.

Gorer (1946*a*) described the pathology of malignant histiocytoma (reticuloendothelioma) of the liver which developed in strain C57BL mice in the Jackson Memorial Laboratory at Bar Harbor. The incidence of this neoplasm reached 15 to 20 percent in mice over 18 months of age. Occasional sporadic cases were seen in other strains. It was Gorer's opinion that this high incidence might have been due to a change in diet. Carcinogenic hydrocarbons increased the incidence and induced this lesion at an earlier age. Gorer noted enlargement of the liver, variable degrees of ascites, and varying involvement of the spleen, mesenteric lymph nodes, and thymus. Microscopically the neoplasm appeared to be multicentric in origin. Phagocytosis by the tumor cells was an outstanding feature. The dominant cell generally appeared to be a mature histiocyte, which in the lymph nodes might be fusiform; in these areas the tumor resembled a spindle-cell sarcoma. One form of growth in the liver was characterized by a disappearance of liver cells and diffuse infiltration of tumor cells in the walls of dilated sinusoids, so that an angiomatous appearance resulted. This variation is noteworthy, since tumors of this structure may be confused with neoplasms of vascular origin. Intravascular thrombi were of regular occurrence in the lungs. Detailed descriptions of the peripheral blood and smears from affected organs were given. A true monocytic leukemia was occasionally found. Syncytial masses of histiocytes were sometimes found in smears from the liver, and evidence of "hyperphagocytosis" by the tumor cells was considered a reliable criterion for diagnosis. Two lines were successfully carried by intraperitoneal and subcutaneous transplantation. By the subcutaneous route, only a small local growth resulted, but later the liver became greatly enlarged. In one line at the 16th transfer, a dramatic transformation occurred and the growth took on the morphology of acute leukemia, with a much shorter survival time of the inoculated mice.

Two recent reports from France described this same tumor type. Roussy and Guerin transplanted several lines of "reticulo-endotheliosis," which they claimed was successfully transplanted in a few cases by a filtrate. They considered some of the cases granulomatous rather than neoplastic. Some neoplasms required 1 to 2 years to develop after inoculation. They attached especial significance to these neoplasms

which they believed formed a link between certain inflammatory and certain neoplastic processes, induced by the action of the same causative agent.

Arvy (1947) described an ovariectomized mouse in which a reticuloendothelial reaction was seen in various lymph-node groups and in the liver and spleen. She thought this justified a diagnosis of leukemia of reticular cells. In the affected mouse the number of megakaryocytes in the spleen was greatly increased.

We have carried two lines of reticulum-cell sarcoma at the National Cancer Institute for periods of 3 and 6 years. These have the characteristics described in reports of other transplantable lines, and show a selective growth in the liver, irrespective of the site at which the transplant was made. Hematoidin crystals have developed with remarkable frequency in the subcutaneous tumor transplants and similar crystals have often been found in sporadic primary spontaneous tumors of this type (Dunn, 1951). The crystals disappear from tissue subjected to routine paraffin embedding and staining with hematoxylin and eosin, yet they are readily demonstrated in glycerine-cleared preparations of fresh tumor tissue. Similar crystals may be found on the surface of other neoplasms, but they seem an integral part of reticulum-cell sarcomas and probably represent a functional activity on the part of the tumor cell in the formation of bile pigment.

Reticulum-cell sarcomas are also notable because they have developed at the site of transplantation of other tumors. A number of reports are available on the development of fibrosarcomas following the transplantation of tumors derived from the mammary gland in both rats and mice, and from the lung in mice. Comparatively few instances of the "transformation" of an epithelial tumor to a reticulum-cell sarcoma are found. Sugiura and Stock reported the development of a reticulum-cell sarcoma from a mammary fibroadenoma in the rat, Dr. D. H. Copeland observed a similar transformation (personal communication), and Andervont and Dunn reported the development of a reticulum-cell sarcoma at the site of a transplanted hepatoma in a mouse, the change developing gradually during the course of several transplant generations.

c) *Characteristic case.*—Type A reticulum-cell neoplasms were found sporadically in different groups of mice at the National Cancer Institute, usually in old animals. Other laboratories have reported a high incidence in the Rb stock and in strain C57BL. Carcinogenic hydrocarbons were reported to increase the incidence and to elicit the tumors at an earlier age.

The most striking clinical symptom is enlargement of the liver and ascites. Primary involvement of the vaginal and uterine wall is common in females, and enlargement of these organs with vaginal bleeding and urinary obstruction may be observed clinically. Invasion of the peripheral blood by tumor cells has not been observed in this laboratory, but others report monocytic leukemia associated with this neoplasm.

At autopsy enlargement of the liver, or the vagina and uterus (fig. 47) may be seen in advanced cases. Infiltration in the liver (fig. 48) is usual

in transplanted lines. In early cases in old mice without symptoms there may be localized enlargement of one or more lymph nodes, or one or both ovaries. Early and extensive involvement of the liver is usual. The spleen, lungs, kidneys, and thymus may occasionally be involved. The tumor tissue is firm and diffusely infiltrating. It is usually white, often shows hemorrhagic foci, and may have a distinctive orange color due to hematoidin. Histologically, the variation in cell forms is an outstanding feature. The basic cell, which is the reticulum cell, has a strongly eosinophilic cytoplasm and a heavily stained basophilic nucleus, but it shows great variation both in the shape and size of the cell and the nucleus, and in the nuclear-cytoplasmic ratio (fig. 49). The cells often appear in sheets of small uniform spindle cells, with heavily basophilic ovoid nuclei and scanty cytoplasm resembling fibroblasts (fig. 50). A characteristic picture is sheets of spindle cells surrounding nests of rounded cells with more abundant cytoplasm, which may lie detached within spaces (fig. 51). These rounded cells often show vacuoles within the cytoplasm. Multinucleated cells are frequent, and may suggest a Langhans-type giant cell. Erythrophagia, and the presence of pigment derived from red-cell destruction within tumor cells, are frequent (fig. 52). Hematoidin crystals can often be found in glycerine-cleared preparations of tumor tissue. Metastasis to the vessels of the lung (fig. 53) or the glomeruli of the kidney are not infrequent in animals bearing transplanted tumors. The tumor in the liver occasionally shows an angiomatous pattern (fig. 54).

On transplantation the tumor grows slowly. I have records of growth extending up to 10 and 12 months. One tumor is reported not to have killed the host until 2 years after inoculation (Roussy and Guerin). Even on subcutaneous transplantation, involvement of the liver is usual, and the local growth may be insignificant from the start or may decrease greatly in size after an initial growth. Transplantation to inbred mice of the same strain is usually successful, and transfer to mice of different strains has been successful in mice in parabiosis. Transplantation to irradiated mice resulted in a great increase in growth rate and wider distribution of the lesion (personal communication from Dr. Charles Congdon).

Cells of the neoplasm have been grown in tissue culture, and the behavior was similar to that of monocytes (Lewis, 1939).

d) Differential diagnosis.—When a reticulum-cell neoplasm, Type A, is fully developed and of typical structure, the diagnosis is easy, for these are histologically distinctive neoplasms. The biological behavior on transplantation also helps to distinguish them from any other type of tumor. Difficulties arise when the neoplasm is small or when it is on the borderline between a granulomatous or neoplastic process. As a rule, the reticulum-cell sarcoma represents a single cell type, the reticulum cell, which is capable of many different expressions, all of which may appear in the neoplasm. Many of the morphologic variations found in these neoplasms are similar to those described by Weiss and Fawcett in chicken monocytes in tissue culture.

Reticulum-cell sarcomas are sometimes found as a single small focus of atypical cells within the medulla of a lymph node, or within the spleen. Even such small foci may be so distinctive that a diagnosis can be made. When, however, there is no involvement of the liver, and a considerable mingling of other forms of cells is found among the hyperplastic reticulum cells, the diagnosis becomes very uncertain. Transplantation may be attempted, but because of the slow growth, a long time is required for an answer.

The conditions with which this neoplasm may be confused are granulomatous hyperplasia, reticulum-cell neoplasm Type B, fibrosarcoma, granulosa-cell tumor of the ovary, and, rarely, hemangioendothelioma and lymphocytic neoplasms.

If a reticulum-cell sarcoma contains an admixture of other cells, particularly lymphocytes and plasma cells, it may closely resemble granulomatous hyperplasia, for both lesions may contain a foreign-body type of giant cell, and large mononuclear cells. Diagnosis of a diffuse reticulum-cell-sarcoma infiltration in the liver may also be uncertain because of a secondary extramedullary hematopoiesis, although these conditions can usually be easily distinguished (fig. 55).

Reticulum-cell neoplasm, Type B (Hodgkin's-like lesion) contains a mixture of various cell types, whereas reticulum-cell sarcoma is composed almost exclusively of a single-cell type, which may show considerable variation in form. Giant cells resembling the Langhans-type cell are infrequent in Type B neoplasms in mice. However, some tumors appear to be intermediate and these borderline lesions cannot be placed in either category. It is probable that many of the Type B neoplasms have been placed in a category with Type A neoplasms in recent reports, and no attempt made to separate them.

If a reticulum-cell sarcoma is composed almost exclusively of a spindle-cell type and originates in the wall of the uterus, it may closely resemble fibrosarcoma. Sections from other organs, however, particularly lymph nodes or liver, usually reveal the polymorphic character of the reticulum cell and some rounded cells with phagocytized erythrocytes or debris are usually found.

I have sometimes found it impossible to differentiate between a reticulum-cell sarcoma and a granulosa-cell tumor of the ovary if this organ alone was involved in the growth, or alone available for study. Great morphologic variation occurs in granulosa-cell tumors, and one variety composed of small spindle cells may be histologically indistinguishable from a reticulum-cell sarcoma primary in the ovary.

An angiomatous type of reticulum-cell sarcoma in the liver closely resembles hemangioendothelioma. This can usually be identified by finding other more characteristic histologic areas.

Occasional cases are found where foci of both reticulum-cell sarcoma and lymphocytic leukemia are found (fig. 56). Undoubtedly a close relation exists between different forms of reticular neoplasms, so the occurrence of more than one histologic type in the same mouse is not unexpected.

Gorer mentions the transformation of one monocytic leukemia line to a lymphocytic leukemia.

e) Comparative pathology and discussion.—Reticulum-cell sarcomas have rarely been reported in wild and domestic animals. Bengston described a case in a cow, and briefly discussed the occurrence of neoplasms of this type in food-producing animals. His case was remarkable for the degree of infiltration of muscular tissue, including the uterus. Close association with reticular fibers, phagocytosis by neoplastic cells, and foci of erythropoiesis within the tumor tissue were noted histologically.

Among other laboratory animals, I have seen one case in a guinea pig (Heston and Deringer, 1952). Nelson and Mario described the lesion in rats and reviewed some preceding reports. Several reports of transplantable lines in rats are cited by Dunham and Stewart. The Yoshida sarcoma probably belongs in this category.

For comparison with neoplasms of this type in man, Furth (1939) is cited. He discussed "a neoplasm of monocytes in mice and its relation to similar neoplasms in man." A transmissible neoplasm had been derived from a mouse that had "granuloma-like infiltrations of the blood forming organs." After successive passages, the strain was transformed into a neoplasm with malignant cells resembling histiocytes, which morphologically were similar to human neoplasms formed by histiocytes. The neoplastic cell was present in small numbers in the blood when the disease was far advanced.

Reticulum-cell sarcomas seem to be relatively less frequent in man than in the mouse, and the neoplasms show morphologic differences in the two species. Neoplasms of the reticulum cell in man have been divided by Gall and Mallory into an undifferentiated syncytial stem-cell type and a more differentiated clasmatocytic lymphoma. Robb-Smith also recognized an undifferentiated or syncytial type, and a type with differentiation to histioid cells. Reticulum-cell sarcomas in the mouse resemble the latter type more closely, and generally show greater evidence of differentiation than the corresponding human neoplasm, as witnessed by phagocytosis of erythrocyte debris, the formation of hematoidin crystals, and variations in size and shape such as the normal reticulum cell exhibits. The neoplastic cell in the mouse thus closely resembles the mature reticulum cell, and the slow growth of this neoplasm may be a reflection of this maturity.

Reticulum-cell neoplasm Type B (Hodgkin's-like lesion)

a) Terminology.—In previous publications we have referred to this lesion as the "Hodgkin's-like lesion." "Hodgkin's disease" has been described in the mouse, and the lesions have some features of Hodgkin's disease in man, but there are differences which make transfer of the term misleading. That the lesion in the mouse is always neoplastic is problematic, but examples having all the morphologic criteria have been successfully transplanted, and the lesion is conveniently discussed under reticulum-cell neoplasms. The terms "lymphogranulomatosis," "reticu-

losis," and "reticuloendotheliosis" have been applied to similar lesions in the mouse. It is probable that lesions of this type have also been included with "histiocytomas" and "monocytomas," but in our experience it is sufficiently distinctive to warrant separation.

b) *Previous descriptions.*—Jobling gave the first description of "Hodgkin's disease" in the mouse and emphasized its resemblance to Hodgkin's disease in man. He described it as a lesion "composed for the most part of cells of the lymphoid type," and "the stroma a delicate reticulum." Important features were many large "endothelioid" cells, some of which were multinuclear, intermingled with lymphocytes and plasma cells. He thought that eosinophils were less frequent in the lesion in the mouse than in man.

Simonds (1925*b*) described lymphogranulomatosis (Hodgkin's disease) in mice, and quotes Ziegler to the effect that "Hodgkin's disease is common in the lower animals." Simonds noted four cases from the Slye stock which he considered to have a close resemblance to lymphogranulomatosis in man. In these four mice only one or two lymph-node groups were involved, and there was no invasion of the capsule. The normal histologic structure was obscured or entirely obliterated by irregular accumulations of large cells, some elongated and resembling fibroblasts, and others with round or indented nuclei which were sometimes multiple. No eosinophils were observed. The spleen was enlarged in all mice and patches of large epithelioid and multinuclear cells were seen. In three mice the liver showed periportal infiltration with lymphocytes and large cells similar to the lesions seen in the lymph glands.

Hill described Hodgkin's disease characterized by an enlarged spleen and some enlarged lymph nodes. She described an eosinophilia in these animals, and the lesion showed a cellular accumulation in which "large and small lymphocytes, plasma cells, eosinophils and occasional eosinophilic myelocytes, a large number of endothelial and endothelioid giant cells, free reticular cells and multinucleated giant cells" were found. The lesions were found in mice that Cherry had inoculated with tuberculous tissue, and 15 were considered typical. Several other cases were of a "mixed type." She emphasized that a thickening of the pylorus opposite the head of the pancreas and involvement of the latter was one of the earliest manifestations of the disease. (Note: This may have been an enlargement of the pancreatic node.) She noted the same admixture of "endothelial" cells and lymphocytes, plasma cells and eosinophils, as have other observers. Areas of fibrosis were described as the disease progressed. The liver contained all these different kinds of cells, usually confined to the sinusoids.

Andersen described cases of "reticulo-endotheliose," some of which may fit into the category of a Hodgkin's-like lesion, although the descriptions and photographs usually resemble reticulum-cell sarcoma more closely.

Furth (1939) in discussing neoplasms of monocytes in mice described certain similarities between these neoplasms and Hodgkin's disease,

although he expressed the opinion that a disease exactly like Hodgkin's had not been found in animals. Both the human disease and the lesion in the mouse had granuloma-like characters. In the mouse there was found a focal and diffuse overgrowth of large mononuclear cells like histiocytes, among and about which were lymphoid cells in variable numbers. Occasional giant cells were found; and eosinophils and plasma cells in a few places. An increase in fibrous tissue was also found. In the course of successive passages, the tumors become more uniform. He considered them unquestionably neoplastic.

In later reports, Hodgkin's disease of mice is infrequently mentioned. Furth and Furth described one case of monocytic leukemia which resembled Hodgkin's disease.

c) *Characteristic case.*—The lesion is rare in young mice, and is most frequent in old mice of certain inbred strains. It is often of sporadic occurrence in hybrids with a long life span. The most distinctive examples of this lesion have appeared in aging strain C57L. It has been studied during a period of several years by Dr. E. D. Murphy, among C57L mice at the National Cancer Institute. Dr. W. E. Heston has reported an incidence of 25 percent in his mice of this strain 18 months of age and older (personal communication). Several transplanted lines have been obtained and this offers an opportunity for further investigation. Much of my information regarding the lesion has been obtained from Dr. Murphy, who plans a publication describing the histology and biological behavior. Clinical features in the strain C57L are enormous enlargement of the mesenteric lymph node (fig. 57), which develops slowly. In other strains, the lesion has seldom been recognized, except in microscopic sections from old, apparently healthy mice killed on schedule, and no clinical features were recorded. No distinctive alteration has been noted in the peripheral blood in C57L mice, and gross observations at autopsy showed enlargement of the mesenteric node. In other strains the primary focus may be some other node or the spleen. Dr. Murphy observed that the Peyer's patches were frequently involved first, but were overshadowed by the mesenteric-node enlargement. The splenic nodules were increased in size, in contrast to lymphocytic leukemia in which the red pulp is most affected. Discrete nodules may be found in the liver. The tumor tissue was firm and white, with little hemorrhage.

On microscopic examination, a more widespread involvement may be found than appears grossly, and the thoracic nodes, bone marrow, and lungs may show the characteristic tissue. In affected lymph nodes the architecture is destroyed, and replaced by the abnormal tissue (fig. 58), yet there may be no great increase in the size, since the process is generally contained within the capsule of the node, which sharply delimits it (fig. 59). The spleen is often involved and areas of neoplastic tissue may replace some normal lymphatic nodules, appearing to proliferate from the center of the nodule (fig. 60). The liver may show focal areas (fig. 65), and less frequently small patches of the abnormal tissue are found in the

bone marrow around the submucosal tissue of the larger bronchi in the lungs, or about larger vessels in the kidney or infiltrating the ovary.

Cytologically, there is a background of large, pale, indistinct reticulum cells intermixed with other cell types (figs. 61, 62). The reticulum cells may have more than one centrally placed nucleus, and multinucleated cells resembling foreign-body giant cells are occasionally found. Small lymphocytes and plasma cells are the most prominent of the other cell types. Eosinophils are not an outstanding feature. A few granulocytes may be present, and occasional small localized abscesses. Scattered pyknotic nuclei and small focal areas of necrosis are frequent. Fibrosis is never a prominent feature. Phagocytosis by tumor cells is never striking.

Transplantation was successful in only a small percentage of trials, and the resulting lines grew slowly (observations of Dr. E. D. Murphy). The morphology of the original tissue was generally maintained, although different generations showed variations in the proportion of different cell types. I have observed several transplanted lines in other mouse strains, and the neoplastic tissue has maintained the morphology of the original during the few transplant generations under observation.

d) *Differential diagnosis.*—The question arises whether this lesion is an entity, and whether separation from other reticulum-cell neoplasms is warranted. The microscopic picture of the Type B lesion is frequently encountered and other observers have thought that it is sufficiently characteristic to require separation. It is the most difficult of all the reticular neoplasms to differentiate because of its resemblance to chronic inflammation, its slow development as a result of which many "early" cases are encountered, and the many borderline cases between other neoplasms, especially lymphocytic neoplasms, and reticulum-cell sarcoma (figs. 63–66). It has features that suggest an inflammatory granuloma, or a metabolic disturbance of the nature of a lipoid storage disease. Inflammatory lesions, however, do not often show the sheetlike background of reticulum cells, and the widespread distribution. Successful transplantation has proved its neoplastic nature.

A patch of the characteristic tissue may be found in a single lymph node, and this may be associated with other forms of reticular neoplasms. Borderline cases between Type B, the Hodgkin's-like lesion, and Type A, reticulum-cell sarcoma, are frequent, but characteristic cases of these two lesions are readily separated. In the Type B neoplasm there is a mingling of different cell types, whereas in the Type A neoplasm the neoplastic tissue consists almost entirely of a single cell form which develops many morphologic variants. The behavior of the Type A and Type B neoplasms on transplantation is dissimilar, and the relative incidence in different strains of mice varies greatly. In some mice, however, a patch of neoplastic reticulum cells reproducing Type A, the reticulum-cell sarcoma, may be found within the Type B, Hodgkin's-like tissue. It is readily distinguished from lymphocytic neoplasms when they are com-

posed of a diffusely infiltrating small lymphocytic cell, but it is difficult to separate it from a lymphocytic neoplasm composed of larger cells which tend to be clumped together. The Type B lesion may also have such a heavy admixture of small lymphocytes that the reticulum-cell background is obscured. A lymph node may be found showing the typical histologic features of the Type B, Hodgkin's-like lesion, confined within the capsule of the node, while an infiltration of neoplastic lymphocytes has extended beyond the capsule and malignant lymphocytes may be found in other organs or tissues of the same animal. The Type C or "Potter lesion" differs in that the reticulum cells appear in sheets separated from the lymphocytes, whereas in the Type B lesions, the cell types are intermingled.

e) Comparative pathology and discussion.—Serious doubt has been expressed that Hodgkin's disease exists in lower animals, and Fox, pathologist to the Zoological Society of Philadelphia, is quoted by Feldman as voicing this opinion. Hodgkin's disease described in the dog by Stalker *et al.* has been considered as sufficiently like the human Hodgkin's disease to warrant the name. McFadyean reported cases in the dog and stated, "If the condition of which these cases are illustrative cannot be enrolled under the head of Hodgkin's disease, it is equally certain that they are not covered by any other recognized term and that a new name is required for them." Medlar and Sasano reported a neoplasm in the rabbit which corresponded closely to Hodgkin's disease in man. A lesion in swine has been described by Forbus and Davis that has many histologic features of Hodgkin's disease and has been the basis of considerable research. Runnells and Benbrook described lesions resembling Hodgkin's disease in a horse and stated that the lesions had a greater resemblance to those of Hodgkin's disease than the authors had ever seen in an animal. Gillman and associates have described lesions called "reticulosis, Hodgkin's-like sarcomata, and histiocytoma" in rats that had received repeated injections of trypan blue. These authors stated that some of the tumors produced by this method in rats were "unmistakably similar to those occurring spontaneously in man, including Hodgkin's disease." They considered the lesion in rats as a satisfactory basis for the study of this group of neoplastic disease. Many aspects of the Gillman reports, however, are unsatisfactory. The Hodgkin's-like sarcoma, for example, is stated to be a variant of histiocytoma, endothelioma, paramonocytoma, or a combination of these three neoplasms of lymph nodes, and Gillman *et al.* (1952) concluded that Hodgkin's sarcoma in the rat did not exist as a separate entity. It was also concluded that the lesions they described were not, strictly speaking, malignant tumors.

The Type B reticulum-cell neoplasm of the mouse, when compared with Hodgkin's disease in man, presents some striking similarities and also many differences. The most important question is whether observations regarding the lesion in the mouse are of any help in understanding Hodgkin's disease in man. Furth (1939) called attention to similarities between Hodgkin's disease in man and neoplasms of monocytes in mice,

particularly when the proliferation of the malignant monocytes was associated with stimulation of other cells. A morphologic similarity is the constant presence of reticulum cells which appear neoplastic, and may even be binucleate, resembling the Sternberg-Reed cell. The reticulum cells in both species are intermingled with lymphocytes and plasma cells. Selected microscopic fields from lesions in the mouse and in man may be strikingly similar. Hodgkin's disease in man, however, presents a variety of forms, from paragranuloma which resembles an inflammatory response, through a variety of types that appear more neoplastic, until Hodgkin's sarcoma is reached, which many pathologists regard as indistinguishable from reticulum-cell sarcoma. The lesion in the mouse does not show this degree of variation and resembles the more neoplastic rather than the inflammatory expressions of the human disease. An important morphologic difference is the relative absence of eosinophils and fibrosis, which are considered by many to be necessary for the diagnosis in man. Reed noted, however, that while an increase in eosinophils was usually present in the human lesion and helped to establish the diagnosis, this was not invariable. Fibrosis, on the other hand, appears to be an integral part of the disease in man as pointed out by Shaw-Dunn.

Similarities and differences are also found in biologic behavior. The lesion in the mouse is very rarely found in young individuals and it shows a steady increase with age, whereas young children are often affected by Hodgkin's disease.

Perhaps the most important similarity between the lesions in men and mice is the difficulty of clearly separating them from inflammatory processes and other neoplasms. Willis has said of the disease in man: "Through Hodgkin's disease, all sarcomas of lymph glands are related," and "Hodgkin's disease is a form of sarcoma of lymphoid tissue, derived from the bipotential stem cells, and exhibiting differentiation predominantly of a reticular cell type with accompanying fibrosis, but also to a variable degree of a lymphoid type." Shaw-Dunn speculated that reticulosarcoma and lymphosarcoma may be considered as one-sided developments of Hodgkin's disease. He stated that the fact that Hodgkin's disease is "the commonest primary lymphadenopathy is surely significant," and he remarked upon the not uncommon finding of microscopic fields of Hodgkin's disease in other lymphoid tissue growths.

Another neoplasm described in man to which the Type B lesion of the mouse has a resemblance is a "mixed" neoplasm in which neoplastic reticulum cells are intermingled with lymphocytes. Warren and Picena found this lesion in human lymph nodes, and separated it from true Hodgkin's disease.

Much of the confusion regarding the lesion in mice may be due to failure to separate several different processes with a similar morphology. The lesions may represent: 1) a preneoplastic lesion which may develop into other more distinctive neoplasms; 2) a metabolic disturbance or "reticulosis" with hyperplasia of reticulum cells; or 3) a true neoplasm seen with predictable frequency in strain C57L mice, and sporadically in other

inbred strains and hybrids. The higher incidence in old mice suggests that a long-continuing disturbance is necessary for its development. The fact that the strain C57L lesion most noticeably affects Peyer's patches and the mesenteric node, indicates that if a primary irritant or carcinogen is responsible, it may have developed in the gastrointestinal tract. It is hoped that further study of this lesion, especially in an inbred strain with an expected high incidence in which transplantation is possible, may give some clue as to its etiology.

Reticulum-cell neoplasm, Type C

a) *Terminology.*—This lesion was described by Potter *et al.* as a preneoplastic lesion in strain C58, and we refer to it in this laboratory as "the Potter lesion." It has never been proved to be neoplastic, but its morphology suggests neoplasia and it is convenient to describe and discuss it along with reticulum-cell neoplasms. It has previously been described as reticuloendothelial hyperplasia, reticulosis, or reticuloendotheliosis. I consider it a distinct entity, worthy of separation from the indefinite lesions usually embraced by the term "reticuloendothelial proliferation."

b) *Previous descriptions.*—Andersen described one lesion among his "Sonderformen" of "lymphomatosis" which is similar to examples of this lesion that I have observed. The lesion described by Andersen was found in the left groin of a 23-month-old mouse. Microscopically it showed strands of indistinctly separated fusiform or round cells, with pale nuclei and faintly eosinophilic cytoplasm, against a background of lymphocytes. In a photograph it was noted that the lesion resembled "Ziegler's large cell hyperplasia" described in man. Andersen considered the lesion to be an early form of reticuloendotheliosis. Potter *et al.* described a similar lesion in old nonleukemic mice of strain C58, and stated that a similar change had not been seen in low-leukemia strains. It appeared in 59 of the 137 strain C58 mice examined by them. The lesion was in general confined to the lymph nodes or liver, appearing only 3 times in the spleen, and never in any other organ. There was gross enlargement of the lymph node in 9 cases, but no preferential involvement of any group. Microscopically the medulla of the lymph nodes appeared to be first affected, although occasionally the entire node was involved. Normal cellular elements were displaced by the hyperplastic growth of reticulum cells. A similar histologic lesion was found in the liver, apparently originating in small nodules of syncytial reticulum in the perivascular reticulum. This lesion was never general for all areas of the liver. Proliferation of lymphoid cells occurred in advanced cases. Carmine injections were never localized in the area of reticulum-cell hyperplasia. Potter *et al.* also observed this lesion in 10 animals on which a series of biopsies were performed before leukemia developed. In the discussion, they referred to the lesion as a "localized reticulum-cell hyperplasia and associated lymphopoiesis" and they suggested that the leukemic lymphocytes in lymphocytic leukemia of strain C58 developed from these foci and the reticulum cells were considered the source of the malignant lymphocyte. The lesion was regarded as definitely preleukemic in strain C58.

c) *Characteristic case.*—This lesion has been seen infrequently in mice from the National Cancer Institute and in sections sent in for diagnosis from outside sources. Typically it appears in older mice, and while it is most common in strain C58, it has also been seen in mice of other strains that have a low incidence of leukemia. A single lymph node, often a superficial cervical or an inguinal node, may be affected and may enlarge to 0.5 cm. or more in size. The lesion is identified by the unique histologic picture, which consists of a central accumulation of pale or slightly eosinophilic reticulum cells, sometimes fusiform, which are surrounded by a mantle of apparently normal small lymphocytes (fig. 67). In the lymph node this tissue may be found only in the medulla, or may replace the entire node. The lesion is less frequently seen in the liver, where it primarily involves the periportal tissues (fig. 68), and is sometimes widely scattered in the parenchymatous liver tissue. It has, rarely, been seen in the liver only and the lymph nodes submitted for histologic study appeared normal. In strain C58 mice with fully developed lymphocytic leukemia in some nodes, other nodes have been found with this lesion only.

d) *Differential diagnosis.*—The lesion must be distinguished from the previously described inflammatory reactions in lymphatic tissue and from reticulum-cell neoplasms, Types A and B. When fully developed, differentiation from other lesions presents no difficulty, for the association of central, pale reticulum cells and bordering lymphocytes has not been found in other conditions. In reticuloendothelial hyperplasia, the reticulum cells are found within the sinuses, the reticulum cells often lie free, and the bordering cells are of various types. Abscesses with a border of leukocytes may have a resemblance, but central necrosis is not found in the Type C lesion. It is distinguished from many granulomatous nodules by the absence of multinucleated giant cells, but an occasional granulomatous lesion, most often seen in the liver, having central, large, often pigmented macrophages and a border of lymphocytes, presents difficulty (fig. 69). In the Type C lesion, however, the reticulum cells appear more syncytial and are not phagocytic. The reticulum-cell neoplasm, Type B (Hodgkin's-like lesion) shows a diffuse intermingling of a large, pale reticulum-type cell and lymphocytes, plasma cells, and other leukocytes. The reticulum-cell sarcoma is composed predominantly of reticulum cells, and multinucleate giant cells are nearly always present.

e) *Comparative pathology and discussion.*—No report of a similar lesion in any other species has been found. "Reticuloendothelial hyperplasia," or "histiocytic sinus reticulosis" in man has considerable resemblance, but lacks the uniformity of the Type C lesion in the mouse. Many features resemble a chronic granulomatous process in man such as Boeck's sarcoid, or tuberculosis, where central monocytic cells may be surrounded by a mantle of small lymphocytes. In the mouse, however, the central monocytic cells are not necrotic and there are no asteroid bodies or multinucleate cells.

In some respects this lesion in the mouse is analogous to giant follicular lymphoma in man, especially in its significance as a premalignant lesion

which becomes transformed to, or actually represents an early stage of, a more aggressive form of neoplasia. This lesion has not been recognized except in histologic sections and no attempts have been made to transplant it; consequently our understanding of it is limited.

5) Plasma Cell

a) Terminology.—Plasma-cell neoplasm, plasmacytoma, and plasma-cell leukemia are terms that have been used in the mouse. Since this neoplasm has not developed in bone marrow, as it has in man, the term myeloma is inappropriate.

b) Previous descriptions.—In a monograph published in 1942 on spontaneous and experimental leukemia in animals, Engelbreth-Holm stated that plasma-cell leukemia, and the related multiple myelomatosis, was the only form of human leukemia not observed in lower animals. In 1951 Rask-Nielsen and Gormsen described spontaneous and induced plasma-cell neoplasia in the Street strain of mice. This strain was known to have a 1 to 2 percent incidence of a type of leukemia that was termed a "stem-cell leukemia" since the authors were uncertain whether the neoplastic cell was a lymphoblast or a myeloblast. Spontaneous plasma-cell neoplasms were also found in 6 cases, or 1.6 percent, of these mice above 14 months of age, and 5 additional examples were found in mice injected with a carcinogenic hydrocarbon. While the carcinogen appeared to accelerate the stem-cell leukemia, no effect was observed on the plasma-cell neoplasms, which were still found only in mice past 14 months of age.

The neoplastic cells were either widely distributed in many nodes and organs or appeared in localized growths. On inspection at autopsy the plasma-cell neoplasms were indistinguishable from stem-cell leukemia, and the nature of the neoplasm was not recognized until histologic sections were examined. Therefore, no transplantation was attempted. An infiltration of the perirenal tissue, splenomegaly, and enlargement of the liver and lymph nodes were notable macroscopic changes in the generalized neoplasms. One plasmacytoma appeared as a slowly growing, subcutaneous nodule in the perineum, one tumor showed metastasis to the lung, one involved the spleen, and one was found in an animal which also had a stem-cell leukemia. Microscopically these neoplasms showed varying degrees of differentiation; some were formed of typical well differentiated plasma cells while others were formed of a cell type resembling a reticulum cell. There were numerous binuclear cells, and occasional irregular giant cells. Mitoses were scarce. Neither the bone marrow nor the peripheral blood was examined.

Two of the tumors were notable in having a pronounced mast-cell infiltration. In one of these, the mast cells appeared atypical and the question of an associated mast-cell neoplasm was raised.

These authors refer to a preceding report by Esmarch of a plasmacytoma in the lung of a Bagg albino mouse which had been injected with methylcholanthrene.

Following the report of Rask-Nielsen and Gormsen, Bichel (1951)

described a transplantable plasma-cell leukemia in the A-k strain, which has a high incidence of lymphatic leukemia. The original tumor appeared in the axilla. On transplantation the tumor became palpable in 6 to 8 days, but grew rather slowly. Microscopically the tumor was very cellular and composed of large mononuclear cells, which were occasionally binuclear. The cells showed all transitional forms from typical plasma cells to cells of a reticulum-cell type. Mitotic figures were abundant. The host animals developed a leukocytosis which was predominantly granulocytic and shortly before death a few plasma cells sometimes appeared in the blood. An infiltration of plasma cells in the perirenal tissue was a conspicuous feature. A marked ascites developed after intraperitoneal injection and growth of the tumor.

I have recently seen lesions in old mice from a variety of inbred strains and hybrids resembling the lesions described by Rask-Nielsen and Gormsen (fig. 70). A distinctive type of plasma-cell neoplasm has also been observed in old strain C3H mice in this laboratory. An ulcerative lesion at the ileocecal junction, showing an infiltration of apparently normal plasma cells, has been seen a number of times. Rarely, the plasma cells appear to undergo a neoplastic change, with an increase in size and hyperchromatism, and occasional binucleate forms are found (fig. 71). The neoplastic cells extend through all coats of the intestine, through the mesenteric fat, and are found in large numbers in the medullary sinuses of the mesenteric node. One of these neoplasms has been successfully transplanted and the percentage of "takes" is small, even though the strain is inbred. The growth rate is slow and irregular. A small tumor which may later disappear forms at the site of subcutaneous transplantation, while the abdominal lymph nodes, the ovaries or the testes, and the kidneys are heavily infiltrated. A modified Russell-body formation often develops, which distinguishes this neoplasm from lymphocytic neoplasms. (A publication describing these neoplasms is being prepared.)

c) Characteristic case.—These neoplasms have been found only in old mice. Two forms have been recognized, a generalized type involving lymph nodes, spleen, and perivascular tissue in the kidney and lungs, and a localized type beginning in the ileocecal area. The morphology of the neoplastic cell is distinctive. The nucleus is eccentric, the cytoplasm contains a clear area (the cytocentrum), and the Giemsa stain shows the violet-colored cytoplasm of the plasma cell. A Russell-body type of alteration may appear.

d) Differential diagnosis.—Plasma-cell neoplasms must be distinguished from plasma-cell hyperplasia of lymph nodes (which is especially frequent in old mice), inflammatory plasma-cell infiltrations, and other neoplasms. The plasma-cell hyperplasia is usually confined to the medulla of the nodes, although centers of the nodule, especially in the spleen, will sometimes show numerous plasma cells. The normal architecture of the node is preserved. Inflammatory plasma-cell infiltrations in other organs are composed of mature plasma cells often accompanied by Russell-body cells. The non-neoplastic hyperplasia usually shows a

mixture of other cell types. When the leukemic plasma cells are of a mature type, however, the diagnosis may be uncertain. Neoplastic plasma cells show abnormalities characteristic of neoplasia, such as large size of the cells, hyperchromatism, and multiple nuclei. The normal architecture of the node is obscured, the cells extend beyond the capsule, and widespread infiltrations are found which follow the general pattern of distribution seen in lymphocytic leukemia. When the neoplasm originates in the ileocecal area, there is a spread to the mesenteric fat and the mesenteric node.

e) Comparative pathology and discussion.—Bullock *et al.* described a transplantable plasmacytoma originating in a rat infested with *Cysticercus fasciolaris*. No other report has been found of a plasma-cell neoplasm in a lower animal.

The neoplasm in mice differs considerably from plasma-cell neoplasms in man, since involvement of the bone marrow and bone has not been reported. However, localized plasmacytomas originating at sites other than the marrow are also reported in man.

It is probable that a larger number of plasma-cell neoplasms of the mouse will be recognized in the future, especially where large numbers of older mice are examined.

6) Tissue Mast Cells

Mast cells are significant in relation to neoplasia under three conditions: 1) infiltration of neoplasms by normal mast cells, 2) hyperplasia of mast cells in tissue exposed to carcinogenic hydrocarbons and irradiation, and 3) neoplasms with either localized tumors (mastocytomas) or generalized invasion of organs and the peripheral blood (leukemia).

1. The infiltration of transplantable splenic tumors by mast cells was described by Bali and Furth. The tumors were probably derived from splenic reticulum as reported by Furth and Krumdieck. Mast cells were present in considerable numbers in all but 1 of 18 of these tumors. Three were successfully transplanted, they grew very slowly, and the infiltration with apparently normal tissue mast cells persisted. Tumors from other sources were stained by the Giemsa method, and mast cells were found in luteomas, pulmonary adenomas, breast cancers, and malignant lymphomas.

Two of the plasma-cell neoplasms described by Rask-Nielsen and Gormsen were heavily infiltrated with mast cells, and in one it was questioned whether a mast-cell neoplasm might be coexistent.

Kirschbaum and Strong reported that atypical cells with basophil granules were a constant feature of one line of myelogenous leukemia, and suggested that the basophilic cells might represent differentiated products of the "malignant" cells, although they were similar in appearance to the tissue mast cells of the normal mouse. Barnes and Furth (1937) observed similar cells in one "myelosis" studied by them. I have observed sarcomas of spindle-cell type in which an extraordinary number of apparently normal mast cells were present. One of particular interest

was found in the adrenal cortex. Fusiform cells often develop beneath the capsule in mice bearing tumors or subjected to some toxic substance. Such an area gave origin to a tumor occupying the entire thickness of the cortex, and mast cells were numerous. I have also observed mast-cell infiltrations in some of the tumor lines described by Earle and Nettleship after exposure of fibroblasts to methylcholanthrene in tissue culture. On transfer of the exposed fibroblasts back to the mouse, a number of tumors resulted. Some of these were carried as continuous lines and others were maintained for only a few generations. One discontinued line showed a heavy infiltration of mast cells, but it was not distinguished in its biologic behavior from other lines in which no mast cells were found.

Sarcomas appear to be more frequently infiltrated by mast cells than do carcinomas.

2. Increase in mast cells after exposure to carcinogens has been described by de Vinyals, Twort and Twort, and Cramer and Simpson. All these observers noted an increase in mast cells in the skin after painting with a carcinogen, and Twort and Twort described a diffuse infiltrative condition of mast cells in the internal organs of one mouse following the application of tar to the skin. Cramer and Simpson discussed the possibility that the mast cells were a protective reaction against the development of squamous-cell carcinomas in the skin. Accumulations of mast cells have been observed frequently in the skin of mice exposed to a variety of carcinogens in experiments conducted at the National Cancer Institute. In irradiated mice Clarkson *et al.* frequently found mast cells in lymph nodes, where they were often associated with free littoral cells containing iron-reacting material. Spargo *et al.* reported an increase in mast cells in the thymus, spleen, and bone marrow in hybrid LAF_1 mice receiving chronic gamma irradiation. Where lymphoma developed, the mast cells were decreased.

3. True neoplasms composed of mast cells are rare in mice.

a) *Terminology.*—Mast-cell neoplasm is the term generally used. The localized accumulations may be called mastocytomas, and more generalized infiltrations having the distribution of lymphocytic leukemia are referred to as mast-cell leukemia.

b) *Previous descriptions.*—Deringer and Dunn described five mast-cell neoplasms observed in mice at the National Cancer Institute, and reviewed preceding reports of mast-cell neoplasms in mice. Lignac reported 2 cases developing after a series of injections of benzol. One case reported by Twort and Twort showed a more extensive infiltration than usually resulted from carcinogenic exposure. Since the publication of the report with Deringer, I have seen many additional cases, and some of these warrant comment. One appeared as a collection of mast cells within the thymus of a year-old strain DBA mouse. The mast cells extended through the thymic capsule and invaded the surrounding fat. One generalized mast-cell leukemia with a marked infiltration in the spleen and liver was seen in a strain C58 mouse (fig. 74). Several neoplasms composed entirely of mast cells have been found on histologic examina-

tion of large collections of subcutaneous tumors, one at the site of a lung-tissue transplant (Heston and Dunn). One noteworthy subcutaneous tumor was first palpated by Dr. M. K. Deringer 6 months before the mouse was killed. The tumor grew to 1.5 cm. in diameter during this period and on histologic section was composed entirely of mast cells, generally fusiform. This is the first direct evidence we have had of the very slow growth rate of these tumors.[8]

c) *Characteristic case.*—It is regrettable that mast-cell neoplasms have not been recognized during the life of the animal, so that more attempts could be made to transplant them. The neoplasms have nearly always been found in old mice in our laboratory, most often in hybrids with a strain A parent. No clinical symptoms have been ascribed to the neoplasms; the growth rate is slow; and only one report of invasion of the peripheral blood was found (Lignac). At autopsy, the tumors were usually localized masses in lymphatic or connective tissue; rarely, the neoplasm was widely distributed, involving lymph nodes, spleen, liver, lungs, and kidneys. Microscopically, the neoplastic mast cell is larger than the normal, it is less heavily and uniformly granulated, and the granules generally do not stain so intensely. The granules may sometimes fuse into masses of metachromatic substance, a change seldom seen in normal mast cells. In larger tumors, the cells may be fusiform, especially toward the center. Mitotic figures are rare. In mast-cell leukemia the cells are widely distributed in a number of organs; large accumulations are found in the red pulp of the spleen, and they may appear singly or in small groups in the lung, the liver, and the kidney.

d) *Differential diagnosis.*—Mast-cell lesions are easily distinguished from other lesions of reticular tissue by metachromatic stains such as toluidine blue. Giemsa is also satisfactory. With hematoxylin and eosin, mast cells may not be recognized, especially if a watery fixative such as Helly's was used. Differentiation from neoplasms infiltrated by mast cells, hyperplasia of mast cells resulting from carcinogens, and hyperplasia from other causes may be difficult.

Infiltration of other neoplasms by mast cells can usually be recognized because the normal mast cells are well differentiated and diffusely scattered. After painting with a carcinogen, large subcutaneous masses composed exclusively of mast cells may be found and the question arises whether these are not actual neoplasms. It seems possible that the mast cells react to the carcinogens as do other cell elements, with hyperplasia which may be succeeded by neoplasia. However, carcinomas developing in the dermis and sarcomas developing in the subcutaneous tissues after painting with carcinogens probably have a greater growth capacity than do mast-cell neoplasms, so that if tissue is transplanted from a painted area, other neoplastic cells rapidly outgrow the mast cells.

The mast-cell neoplasms encountered in the mouse grow slowly, and have a low degree of "malignancy," but the accumulation of many ab-

[8] In a personal communication, Dr. L. Foulds stated that he had observed a successfully transplanted mast-cell neoplasm carried for several generations.

normal cells at sites in which few mast cells are ordinarily found is more easily explained as resulting from neoplasia than from any other cause.

e) Comparative pathology and discussion.—The comparative pathology of lesions of mast cells in different species of animals is confused by our inability to make a sharp distinction between mast-cell hyperplasia and neoplasia. Kelsall and Crabb noted an increase in mast cells in the thymus of X-irradiated hamsters. In rats exposed to methylcholanthrene and benzpyrene by Holmgren and Wohlfart, the resulting fibrosarcomas regularly showed a marked infiltration with mast cells. This infiltration continued in some tumors through several transplant generations, and then appeared to be declining. The authors believed that the cells developed locally in the connective-tissue stroma, and were probably a reaction against the tumor growth. No mast-cell neoplasms have been reported in the rat, but I have examined sections of lymph nodes which were increased in size and in which the normal cells were replaced by mast cells larger than normal. Symmers and Thomson described mast-cell lesions, which they did not consider neoplastic, in a ferret.

Deringer and Dunn cite references to mast-cell tumors in other species. Mastocytomas are relatively frequent in the dog, and have been described by Bloom. I have examined a number collected by Dr. W. H. Eyestone. The neoplasm in the dog contains numerous eosinophilic granulocytes and fibroblasts, and the mast cells are less closely packed than in neoplasms in the mouse. Mast-cell leukemia in the cat, with widespread distribution such as is sometimes seen in mice, was described by Lillie, and Bloom stated that the condition was not infrequent in cats.

It is doubtful that mast-cell neoplasms exist in man. A marked increase in blood basophils often accompanies chronic granulocytic leukemia (Forkner). Dense accumulations of mast cells occur in urticaria pigmentosa, and Ellis described a case in an infant with an increased number of mast cells in the lymph nodes, spleen, liver, and bone marrow. He noted points of resemblance to cancer, and a parallelism to mast-cell neoplasms in the dog.

7) *Unclassified and Miscellaneous*

When an attempt is made to sort any group of pathologic lesions, some are nearly always found that cannot be fitted into any established category. The failure of the classification adopted to include all lesions may be because some lesions of diverse and uncertain origin do not obligingly conform to the criteria that have been selected for delimiting a particular group. If a sufficient number of lesions of the nonconforming type are found, and an opportuinty is given for studying them, a separate category may be warranted and a provisional diagnosis made. In addition to lesions of this nature about which uncertainty exists as to their proper place in a histogenetic scheme, lesions of an unusual type may be encountered about which there is no particular problem as to origin, but which are too few in number to warrant a special category and therefore

require an individual description. These tumors which either cannot be diagnosed or are few in number are grouped as "unclassified and miscellaneous tumors." Other lesions may not be classified because postmortem changes have obscured histologic details, or the microscopic sections may be unsatisfactory for some other reason.

Previous descriptions of tumors in which a decision as to the cell of origin was not made have been given by Furth and Krumdieck and by Barnes and Furth (1940). Furth and Krumdieck described a tumor that was usually single and circumscribed and was composed of polygonal cells with finely granular, slightly acidophilic cytoplasm. The cell of origin was considered by the authors to be unlike the usual malignant reticulum cell hitherto described in tumors of men and animals. The most likely precursor was thought to be a reticulum cell forming the reticular framework of the spleen. Some of these tumors were induced by the introduction of benzpyrene into the spleen, but others were spontaneous in the Ak strain. Spontaneous tumors of a similar type were found in an equal number of stock Rb mice. The tumors were considered benign, although in a later paper Bali and Furth reported that they were successfully transplanted. This later report also stressed the epithelial character of the tumor cells and noted a resemblance to carotid-body cells. In the later paper, the presence of many mast cells in the splenic tumors was described.

Barnes and Furth (1940) described another unusual transplantable tumor. Evidence was presented that the neoplastic cells were related to reticular or endothelial stromal cells of the spleen and lymph nodes. The tumor cells varied in size, many contained a central area of lightly staining cytoplasm, and some were multinucleated.

Two unusual tumors of reticular tissue observed at the National Cancer Institute are pictured (figs. 75, 76, 77).

One report of an eosinophilic myeloid leukemia was given by Lignac in a group of mice exposed to benzol. An increase in eosinophilic cells appeared in the peripheral blood, and there was an eosinophilic infiltration in various tissues.

No report has been found of congenital leukemia and no report of polycythemia in a mouse.

VI. Lesions Found in Mice of Various Strains and Types of Hybrids at the National Cancer Institute

Collections of microscopic slides taken from various groups of mice autopsied at the National Cancer Institute have been reviewed (table 4). Accurate data were kept on the ancestry of the mice, the age and sex, and any experimental treatment. All mice in the group had been autopsied, but as a rule sections for microscopic study were taken only when some abnormality was apparent on gross examination. A comprehensive investigation to determine the incidence of various lesions of the reticular system had not been planned, and the results represent an estimate of

the relative frequency of the lesions. Data from some of these groups have been or will be published in other communications. The name of the investigator who permitted use of the slides is given.

The lesions regularly listed were lymphocytic leukemia, reticulum-cell neoplasms Types A and B, other less frequent neoplasms, and mesenteric disease. Except for mesenteric disease, only one lesion was listed for any one mouse, but where mesenteric disease occurred with a neoplastic process, both were listed. Mesenteric disease was also the only lesion in which a gross observation alone was sometimes accepted as sufficient for diagnosis. All other conditions were confirmed by microscopic examination.

Many of these collections of slides had been examined at periods of several years to several months previously, but they were re-examined without consulting the original diagnosis. It was noted that a high percentage of difference between these two diagnoses, sometimes up to 20 percent, was found in some groups of mice. The differences nearly always involved a choice between reticulum-cell neoplasm, Type B (Hodgkin's-like lesion) and lymphocytic neoplasm, or Type B and Type A reticulum-cell neoplasms, which is evidence of the indistinctness and overlapping in the Type B lesion.

Discussion.—The following points regarding table 4 are worthy of note:

1. Age incidence.—Lymphocytic neoplasms generally appeared at an earlier age than any other form of neoplasm. Reticulum-cell neoplasms, Types A and B, and rarer forms of neoplasm appeared in older mice.

2. Reticulum-cell neoplasm, Type B, appeared frequently in strain C57L hybrids, and its incidence was not related to any other form of neoplasm. It was generally more frequent in females, but this point was difficult to establish, since more female mice were allowed to reach an advanced age.

3. In the only hybrids (C3Hf $\times$ C58)F_1 in which there was a preponderance of males, the females had lymphocytic neoplasms at an earlier age than the males.

4. Mesenteric disease was frequent in strain C3H and its hybrids. The condition was not related to any neoplastic process.

VII. Significance of Neoplastic Lesions of the Recticular System in Mice in Relation to Disease in Man

The question now arises as to what significance these pathologic changes in the mouse may have in relation to neoplastic disease of the reticular system in man. Furth (1952) discussed some aspects of this problem in a recent address. So much similarity exists both in morphology and biologic behavior that it is reasonably certain that they represent the same fundamental process, and that general deductions can safely be made from the findings in mice to the disease in man. It is proper, therefore, to demand that any theory as to the etiology and pathogenesis of leukemia in man shall recognize the neoplastic nature of the disease, since the process

TABLE 4.—*Incidence of reticular*

Contributor	Type of mouse			Treatment	Type of neoplasm or lesion					
	No. and sex	Strain	No. over 18 mos.		Lymphocytic		Reticulum cell Type A		Reticulum cell Type B	
					Percent	Age†	Percent	Age†	Percent	Age†
Heston....	100♀	(C3Hf × C57BL) F_1	88	0	4	28	4	29	10	29.2
Heston....	99♀	(C57BL × C3Hf) F_1	84	0	4	25	9	27	6	28
Heston....	101♂ 150♀	AHR-HRBC	200	0	0.4	21	2.8 (7♀)	21	1.2 (2♂ 1♀)	26
Heston....	175♂ 202♀	AWA-WABC	190	0	0.8	21	1.6 (6♀)	22	2.6 (2♂ 4♀)	20
Deringer...	33♂ 74♀	HR*	66	0	8.4	16	6.5	20	11.2 (1♂ 11♀)	20
Deringer...	74♂ 87♀	HR	23	400 r X ray at birth	4.3	10	0	.....	2.5	17
Deringer...	42♂ 48♀	HR	0	Repeated MCA painting	0	.....	0	.....	0	.....
Deringer...	19♂ 28♀	HR	0	Repeated MCA injections	2	12	0	.....	0	.....
Deringer...	17♂ 34♀	HR	35	Repeated ether painting (MCA control)	7.8	13	5.8	19	23.5	20
Deringer...	222♀	(C57L × A) F_1	125	0	2.2	19	0.9	18	10.3	23
Deringer...	153♀	C3Hf	106	0	3.9	20	0.6	26	3.9	26
Deringer...	104♀	A	21	0	3.8	7	1	20	3.8	20
Law.....	37♂ 40♀	(AKR × NH) F_1	45	0	37.0	15	0	.....	11 (9♀)	 16
Law.....	60♂ 80♀	(NH × AKR) F_1	84	0	22.0	17	3	24	9 (1♂ 12♀)	22
Law.....	58♂ 64♀	(C58 × C3Hf) F_1	73	0	33.0	16	3	21	7 (7♂ 2♀)	24
Law.....	82♂ 43♀	(C3Hf × C58) F_1	91	0	38.0	20	3	22	11 (9♂ 4♀)	24

*No difference found in haired and hairless, hence combined for reporting.

in mice is readily transplantable and fulfills all the requirements for a neoplasm. Many theories which have been arrived at after observation of the disease in man only must be viewed with skepticism. Among these are the belief that it may be caused by an organism, that it may be an excessive hematopoietic response of a non-neoplastic cell, that it may be a metabolic disturbance with failure of maturation and dependent upon the deficiency or excess of some metabolite or food factor, or that it may be a failure of a normal mechanism for the removal of leukocytes. Each of these theories can be defended if the information obtainable from human cases only is relied upon. But if leukemia is acknowledged to be similar in all mammalian species and not a reaction peculiar to one species only

lesions from different groups of mice

Type of neoplasm or lesion (*continued*)			Remarks
Mesenteric disease		Others	
Per-cent	Age†		
14	26	0	Advanced age of onset, even of lymphocytic leukemia. High incidence mesenteric disease.
11	30	0	Generally advanced age, lymphocytic leukemia first to appear. High incidence mesenteric disease.
0.4	20	0	Low incidence of reticular neoplasms and mesenteric disease. Relatively early age.
1	19	0	Neoplasms at an earlier age than C3Hf hybrids. Reticular neoplasms more frequent in female.
3.7	25	2 granulocytic—age† 21.	Control strain hairless mice had reticular neoplasms at a comparatively early age. X ray at birth decreased age, although total incidence was reduced and life span shortened. High incidence of Type B reticulum-cell neoplasm in mice painted with ether is unexplained.
0		1 granulocytic—age† 15.	
0			
1.9	23		
0			
0			High incidence of reticulum-cell neoplasm, Type B, may be influence of the strain C57L parent.
43.1	23	2 plasma-cell—generalized—age† 22.	High incidence of mesenteric disease.
0			Early age of lymphocytic leukemia. Low incidence of other neoplasms may be due to short life span.
0		1 granulocytic—16 mos.: 2 plasma-cell—age† 18.	High incidence of lymphocytic leukemia. Relatively high incidence of reticulum-cell neoplasm, Type B, all in females.
0			Lymphocytic leukemia in smaller percent and more advanced age than preceding.
5	25	1 granulocytic—14 mos.; 1 plasma-cell—age† 13.	Lymphocytic leukemia: 18♂ age† 19; 33♀ age† 13; early development in female. Low incidence of mesenteric disease in this C3H hybrid.
8	26		Lymphocytic leukemia—29♂ age† 21; 9♀ age† 15; early development in female.

†Average age in months.

and unique in man, then any theory applied to human leukemia must be supported by experimental work with mice.

It has been proposed by pathologists working with human lymphocytic leukemia and allied neoplasms that these diseases represent a modification and variation of a single disease process. To quote Willis: "Recognition of this [that the components of lymphoid tissue are derived by divergent differentiation from primitive mesenchymal stem cells] will enable us to see the several named types of tumours, not as rigidly distinct species, but as related variants of a single species and the names we apply will then be used as convenient descriptive labels and not as bones of contention over histogenesis. We will cease to be surprised that Hodgkin's

disease merges with lymphosarcoma on the one hand and with 'reticulosarcoma' on the other." Custer and Bernhard have prepared a chart showing the interrelationship of lymphomas, and indicated the transitions from one form to another. They concluded that there was "a striking fluidity in histologic pattern, with transitions and combinations that could be best interpreted as indicating a single neoplastic entity having a number of variants."

A similar point of view is applicable to neoplasms of the reticular system in mice. Failure to separate exactly a considerable percentage of reticulum-cell neoplasms, Type B, from lymphocytic leukemia and reticulum-cell neoplasm, Type A, in the mouse is in keeping with the experience with human neoplasms. It is somewhat similar to the problem of separating neoplasms of the mammary gland in the mouse. Here the tumor may appear organoid with different elements of the gland participating in different degrees in the formation of the tumor, so that separation into isolated types is never exact, but only a useful rough index that is of value when large numbers of tumors are available. Reticular neoplasms in mice are derived from a complex but closely related system, to which many cell forms belong. Various elements may be preponderant in a neoplasm, but wider experience with the histogenesis of neoplasms indicates that they are seldom, if ever, made up of "pure" cells of one type only.

The age incidence of these diseases in the mouse is of interest in comparison with the age incidence in man. Of all the leukemic diseases, lymphocytic leukemia appears most often in children and is acute and rapidly progressive, while reticulum-cell sarcoma generally appears in an older age group and may be of long duration. In mice, lymphocytic leukemia generally appears at a younger age. It may have a high incidence in certain inbred strains, and it kills quickly, especially if accelerated by a series of animal passages. Reticulum-cell neoplasms, on the other hand, develop in old mice, grow slowly, and even after repeated transfer they may not accelerate.

The claim that an antagonism exists between lymphocytic and granulocytic leukemia in the human being has been recently supported by Dausset and Schwarzmann. The argument is based principally on the difference in age incidence, since lymphocytic leukemia is more frequent in early and late life, while granulocytic leukemia is found in the middle years. The idea of an antagonism is not borne out by incidence studies in mice, since granulocytic leukemia has appeared in the same strains as those in which lymphocytic leukemia is common. It is true that in mice a high incidence of lymphocytic leukemia developing at an early age and leading to early death prevents the development of other forms of neoplasm at a later time. Thus a strain with many lymphocytic neoplasms may have relatively few of other types.

As Storti pointed out, experiments with mouse leukemia are not in agreement with the opinion that acute and chronic leukemia in man may represent two different processes, the former an infection and the latter a

neoplasm. The same strain of transmissible myeloid leukemia was transplanted by him under different conditions and resulted in different periods of survival in the host animals. This he interpreted as evidence that no essential difference is to be found in the two conditions in man.

The fact that leukemia in mice can be transmitted by only a few cells injected at one site should be given weight in the controversy as to whether human reticular neoplasms may start from a single focus or represent a systemic disease with multiple sites of origin. In the spontaneous disease in mice, however, more than one lymph node is usually found affected even at the earliest stage, and in mice of a high-leukemia stock, removal of affected nodes will not prevent a generalized form of the disease. In those strains, however, in which the thymus is the most frequent primary site, thymectomy will both delay and often prevent the development of leukemia. In this laboratory we have removed transplanted masses of reticulum-cell sarcoma a short time after subcutaneous injection, but this rarely prevented the development of liver metastasis. Regarding the question of unicentric versus multicentric origin, it seems probable that unicentric origin is possible but the rapid spread of leukemic cells makes their containment at a single site for any length of time improbable. Moreover in an animal liable to leukemia because of genetic or other factors, multiple primary sites are frequent.

The study of leukemia in inbred strains of mice offers some points for consideration as to the etiology of leukemia in man. A strong hereditary predisposition is present in some strains but the resulting incidence can be increased or decreased by extraneous factors. I know of no strain of mouse in which leukemia has never been reported, so there is no absolute resistance in any type of mouse; but in the relatively resistant strains, the factor of age is of great importance, and the disease occurs in older mice.

The individuality that appears in various transplanted lines of leukemia in the mouse may be of significance in regard to human leukemia. The transplanted line, so long as it is kept in the same inbred strain under the same conditions, reproduces with remarkable regularity the same effect in every mouse to which it is transferred. It may become rapidly generalized, or remain localized, and it may show a particular predilection for certain organs. Even the response to chemotherapy will vary with different lines. Clinicians are puzzled that cases of leukemia in which the morphologic criteria seem to be identical, and where the patients are of similar constitution, may yet run different clinical courses and respond variously to treatment. This variability corresponds to the lack of uniformity in leukemia lines in the mouse. The study of the human case ends with the death of the human host, no reliable controls can be used, so that all the characteristics of the "line" can never be explored.

The acceleration shown by some lines of mouse leukemia may be paralleled by cases of human leukemia which may show increasing malignancy after relatively slow progression, or remission.

Many other points of comparison between the diseases in the two

species might be suggested. An understanding of the neoplastic process in the mouse should be of help toward an understanding of the problem of leukemia in man.

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PLATES

289972—54——17

All photomicrographs are stained with hematoxylin and eosin, unless otherwise stated

PLATE 91

Cells from the peripheral blood of the mouse. Smears were stained with Wilson's stain (modified Wright's stain), and photographed × 1,000. (Courtesy Dr. Clarence Velat, National Cancer Institute.)

FIGURE 1.—*Newborn mouse.* Normoblast in center, marked polychromasia, large size of erythrocytes, moderate anisocytosis, and uniform hemoglobinization of erythrocytes.

FIGURE 2.—*Adult mouse.* Uniform ring-shaped nucleus and granularity of eosinophilic granulocyte (*above*) in contrast to the irregularly lobulated nucleus and lack of visible granules in the two neutrophilic granulocytes (*below*). A clump of platelets in upper left. Hemoglobinization of erythrocytes is irregular, and more concentrated at margin.

FIGURE 3.—*Adult mouse.* Large lymphocyte, with heavy chromatin clumps in nucleus, a pseudonucleolus and relatively large amount of cytoplasm. A small lymphocyte lies above. Irregular hemoglobinization is especially evident.

FIGURE 4.—*Adult mouse.* Three lymphocytes lying above a monocyte. The monocyte has a lobulated nucleus with generally finer strands of chromatin, resulting in lighter staining, than the nucleus of the large lymphocyte in figure 3. On direct microscopic inspection the cytoplasm of the monocyte has a uniform finely granular appearance, while the cytoplasm of the large lymphocyte usually appears homogeneous with variation in staining density.

FIGURE 5.—*Adult mouse.* Neutrophilic granulocyte above, lymphocyte on left, a clump of platelets, and eosinophilic granulocyte below. A few platelets are superimposed on erythrocytes. The blue irregular mass in the center is probably an artefact.

FIGURE 6.—*Mouse with lymphocytic leukemia.* Strain A. Abundance of neoplastic cells reproducing developmental stages of the lymphocyte. Two "prolymphocytes" are seen in lower right.

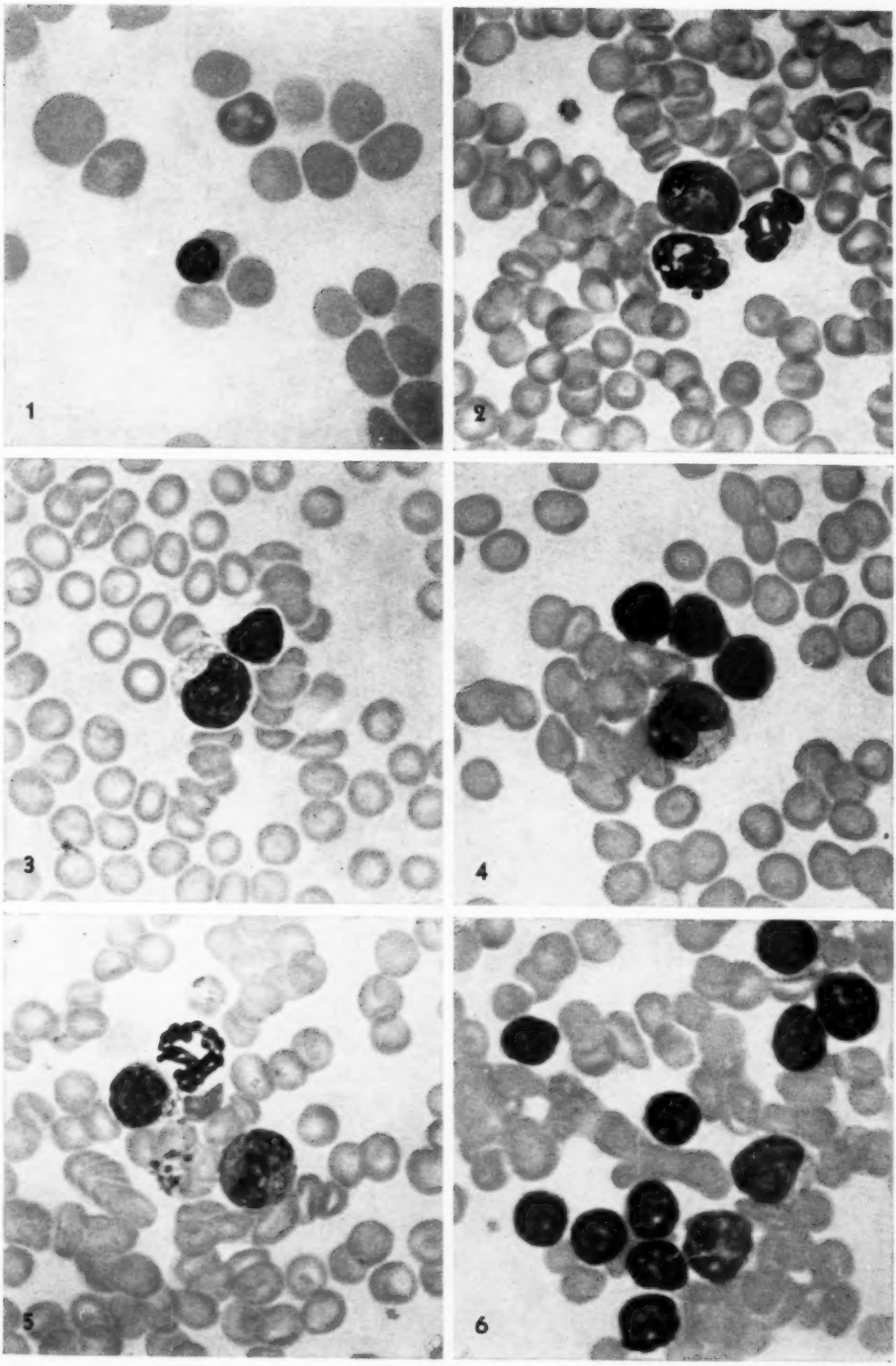

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PLATE 92

FIGURE 7.—Diagrammatic sketch to show location of lymph nodes, spleen, and thymus in the mouse.

1. Superficial cervical nodes. Lateral node is partly obscured by parotid salivary gland tissue.
2. Deep cervical nodes.
3. Thoracic nodes lying behind the thymus.
4. Axillary node.
5. Brachial node.

A. Thymus.

B. Spleen.

6. Pancreatic nodes lying at margin of pancreas and behind stomach.
7. Renal nodes.
8. Mesenteric node.
9. Inguinal node.
10. Lumbar nodes.
11. Caudal node.
12. Sciatic node lying in the back below sciatic nerve.

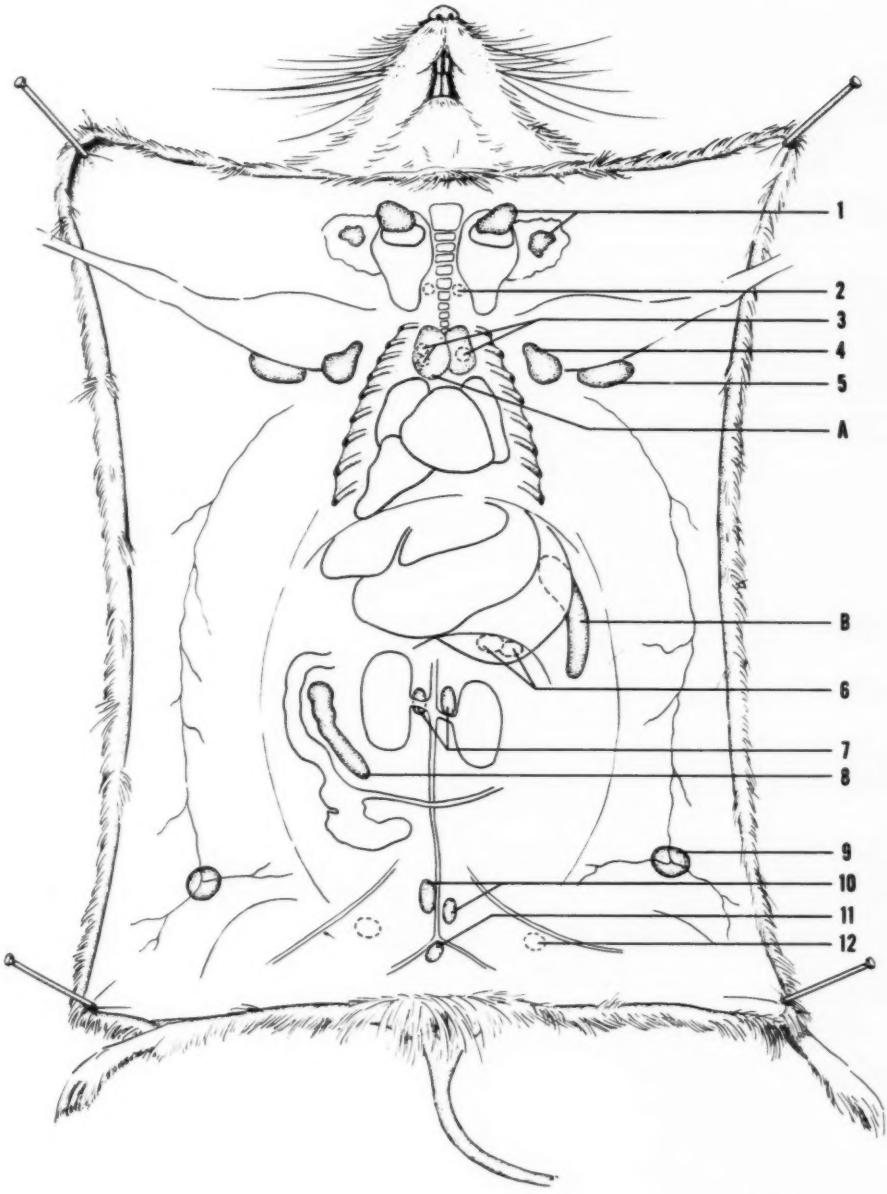

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PLATE 93

Sections of spleen from various inbred mice. The sections were prepared by a modification of del Rio Hortega's method for demonstrating reticuloendothelial cells. All mice were males about 3 months of age. (*Courtesy Dr. Oscar Duqué.*)

FIGURE 8.—Strain C3Hf mouse. The central area of the follicle is surrounded by a well developed "perifollicular collar" of reticuloendothelial cells, one or two cell-layers thick. Peripheral to the collar is a "perifollicular halo." × 84

FIGURE 9.—High-power view of reticuloendothelial cells from the red pulp of the spleen of a strain C3H mouse. × 390

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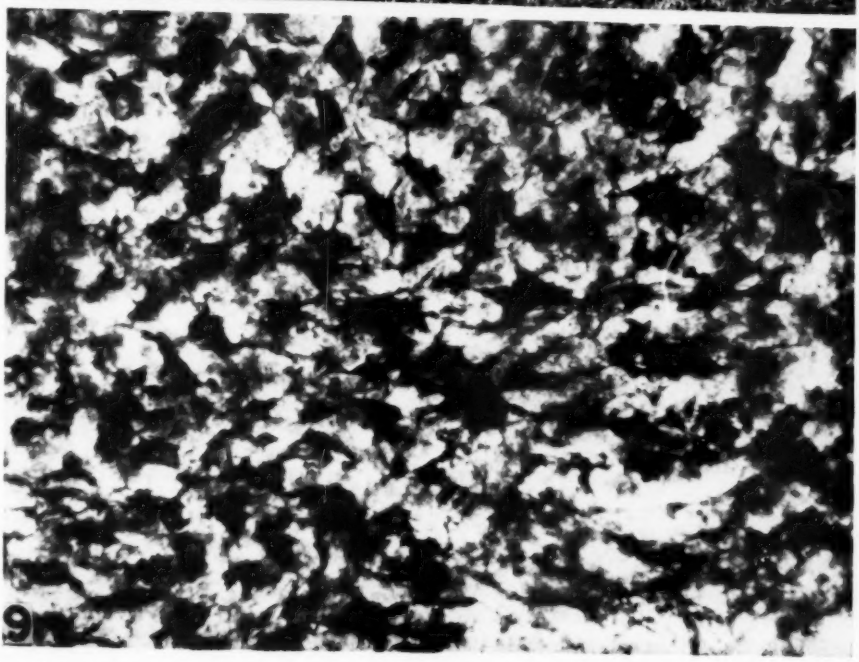
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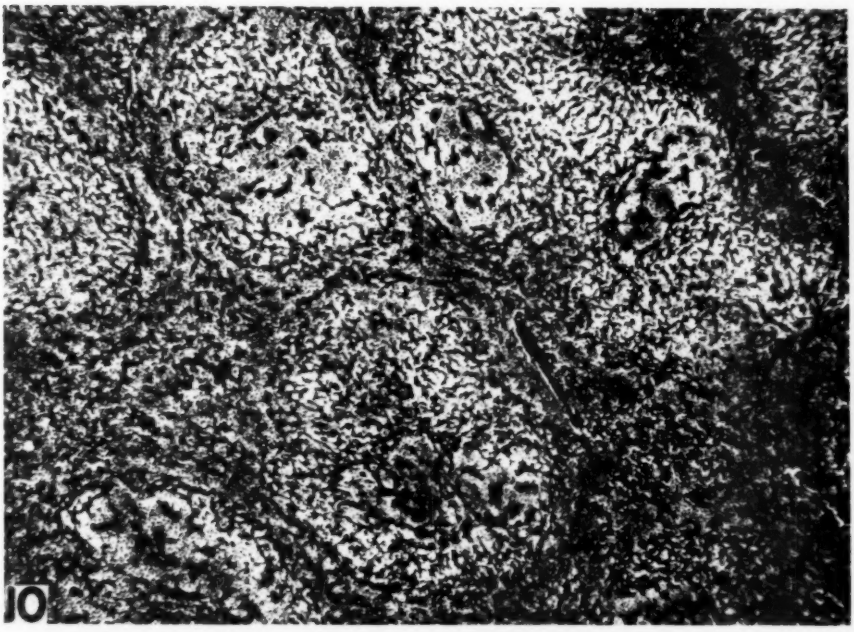
PLATE 94

Sections of spleen from various inbred mice. The sections were prepared by a modification of del Rio Hortega's method for demonstrating reticuloendothelial cells. All mice were males about 3 months of age. (Courtesy Dr. Oscar Duqué.)

FIGURE 10.—Strain C57BL mouse. Some variation appears in this strain but the most common type is pictured here. Both the perifollicular collar and the perifollicular halo are less sharply demarcated than in strain C3H, and the perifollicular collar is missing in some places. × 84

FIGURE 11.—Strain A mouse. The perifollicular collar varies in this strain, and in some animals it is missing. The follicular reticuloendothelial cells are numerous and well developed. × 84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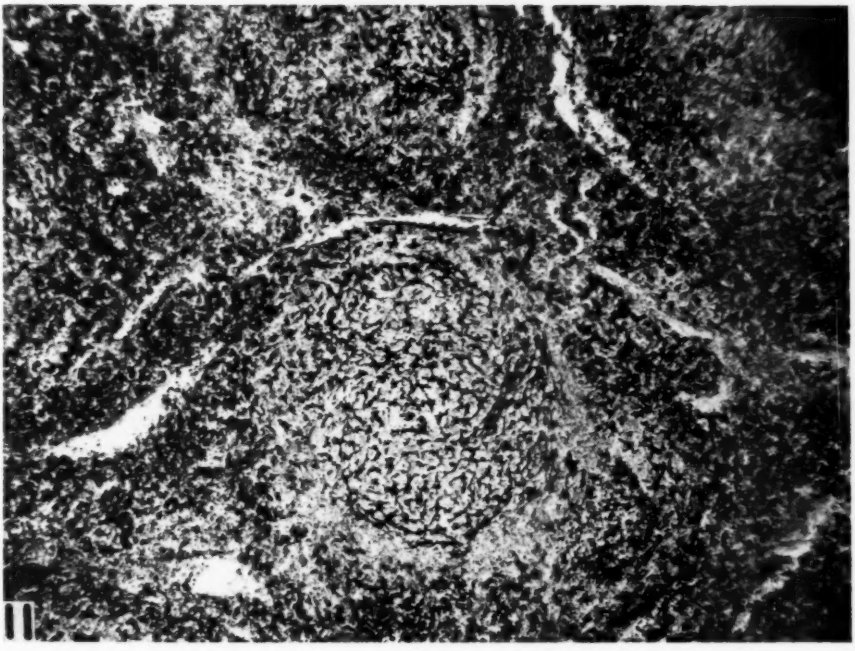
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PLATE 95

Prussian-blue reaction on sections of spleen of inbred mice.

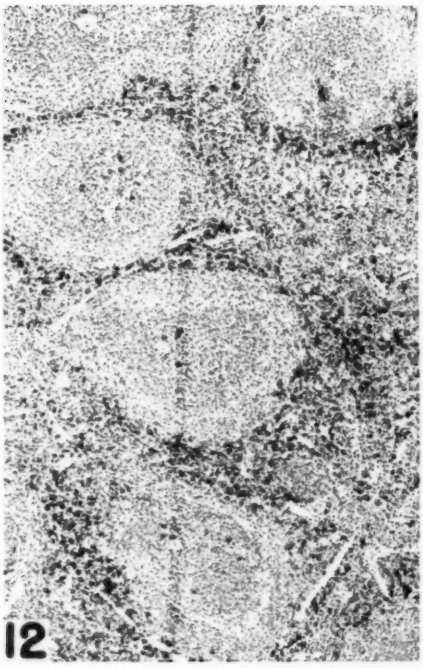
FIGURE 12.—Strain BALB/c male, 12 months of age. Note concentration of iron-positive material in perifollicular area, paucity of iron in centers of nodules, and abundance in red pulp. × 70

FIGURE 13.—Strain C58 male, 12 months of age. Note absence of perifollicular iron, and concentration of iron-positive substance in center of nodules. × 70

Accumulation of small lymphocytes in endothelial-lined spaces in lymphatic tissue of mouse.

FIGURE 14.—Center of splenic nodule from spleen in a strain C3Hf male, 4 months old, which received a toxic dose of aureomycin by stomach tube 54 hours previously. This is a recovery stage after "shock." Note lymphocytes in endothelial-lined spaces. × 190

FIGURE 15.—(C3Hf × C58)F_1 male, 19 months of age. Endothelial-lined space crowded with small lymphocytes in a lymph node which is the site of a reticulum-cell neoplasm, Type B (Hodgkin's-like lesion). × 280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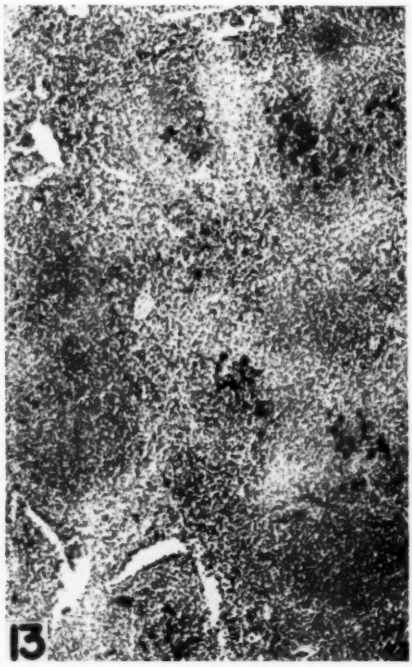
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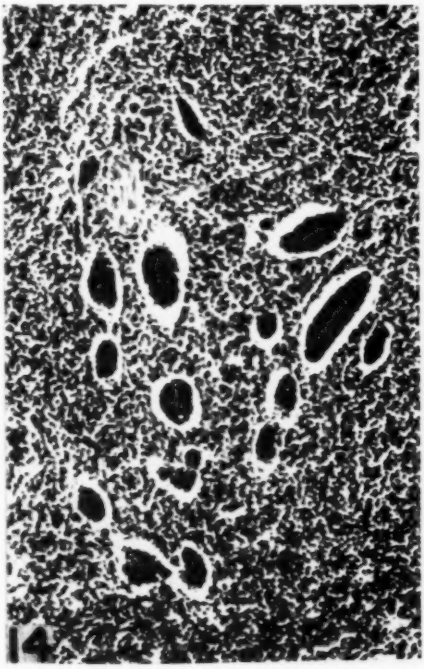
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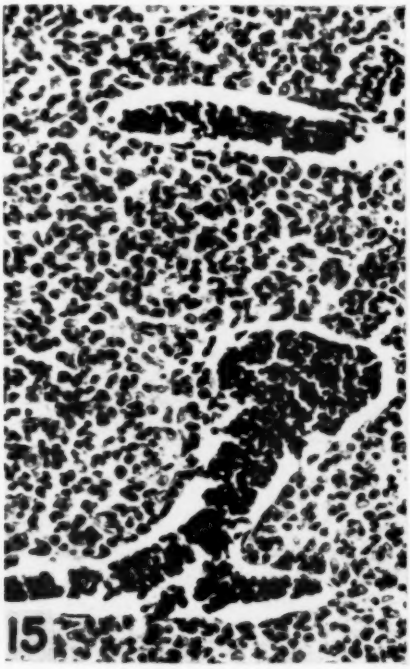
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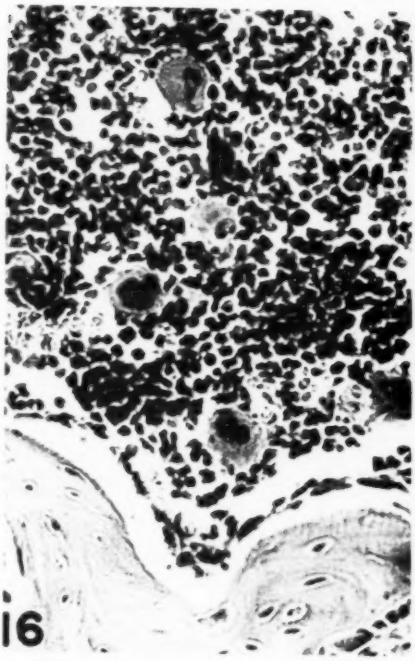
PLATE 96

FIGURE 16.—Strain C3H female, 3 months old. Normal bone marrow from sternum. Marrow cells completely fill space. Megakaryocytes, immature granulocytes, and nucleated erythrocytes are present. × 350

FIGURE 17.—Strain C3H female, 3 months old. Thiamine deficiency. Bone marrow from sternum. Greatly dilated vascular channels filled with erythrocytes replace most of marrow cells. × 350

FIGURE 18.—Strain C3Hf female, 3 months old. Marrow from middle third of tail, a fatty marrow with few hematopoietic cells. × 250

FIGURE 19.—Lymphatic tissue composed chiefly of lymphocytes, occupying space which is filled with parathyroid tissue on opposite side. Slight granulocytic infiltration in thyroid gland. × 75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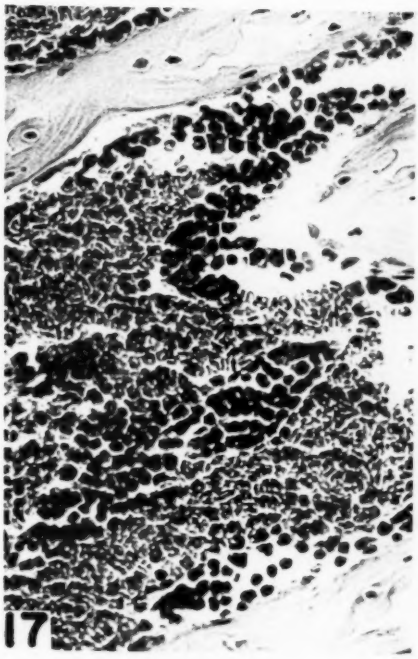
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PLATE 97

Photographs of important lymph nodes in the mouse. Strain C58 animals from which these were made had early generalized lymphocytic neoplasm, and all nodes were enlarged. Magnification × 2

FIGURE 20.—Superficial cervical nodes (*C*) showing relation to salivary gland. The axillary node (*A*) lies within the axilla, and the brachial node (*B*) below belly of biceps muscle.

FIGURE 21.—Mesenteric node (*M*) lying in mesentery of ascending colon.

FIGURE 22.—Lumbar nodes (*L*) lying on either side of the vena cava. The caudal node (*C*) lies below the bifurcation.

FIGURE 23.—Dorsal view of sciatic nodes lying slightly below the point of emergence of the sciatic nerve.

C
A
B
20

M
21

L
C
22

23

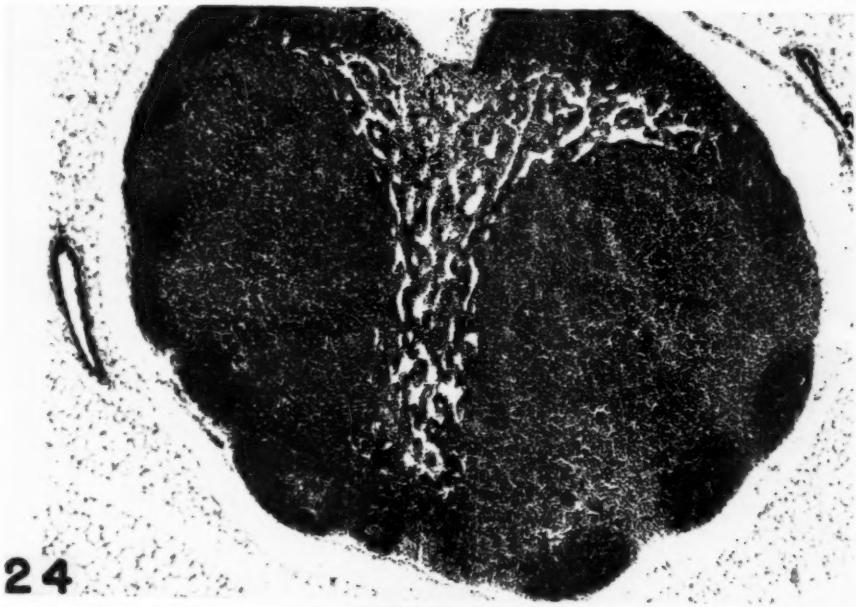
PLATE 98

Lymphatic tissue from normal mice.

FIGURE 24.—Strain DBA female, 3 months of age. Inguinal lymph node, showing a peripheral cortical zone with lymphatic nodules indistinctly outlined. Tissue which is composed of medullary cords and sinuses extends from the hilum and partially separates the node into two parts. Tissue principally composed of loosely packed lymphocytes lies between the cortex and medulla, and has been referred to as the "intermediate zone." × 75

FIGURE 25.—Strain BALB/c male, 6 weeks of age. A lumbar lymph node showing cortical and medullary tissue at opposite ends of the node. The inguinal and the lumbar node represent slightly different structural patterns in lymph nodes of the mouse. × 60

FIGURE 26.—Strain BALB/c male, 12 months of age. Peyer's patch, showing interruption of the muscularis mucosa, and absence of villi immediately above the nodules, which are covered by a columnar epithelium containing nuclear debris. Note that a mass of epithelial tissue is found at considerable depth in the lymphatic tissue. × 84



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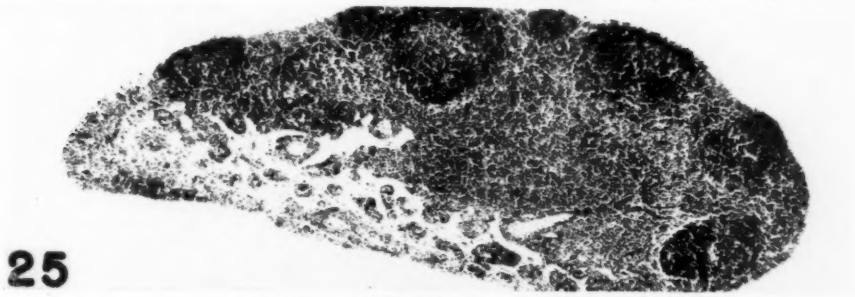

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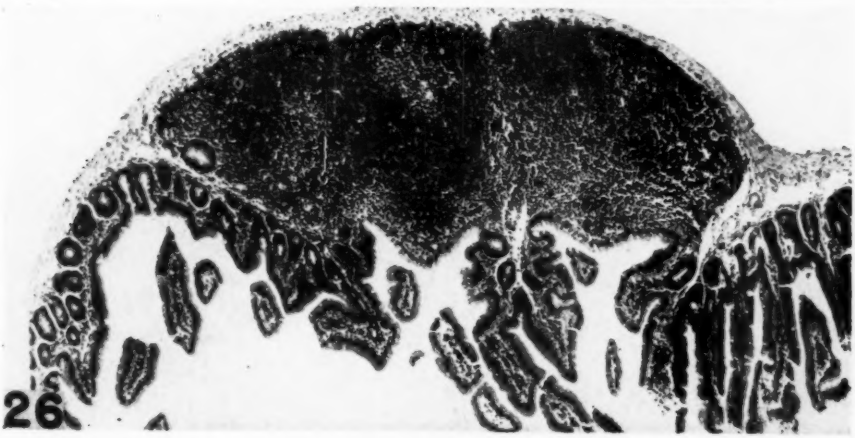

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PLATE 99

Mesenteric disease. Mesenteric lymph node from strain C3H male, 18 months of age.

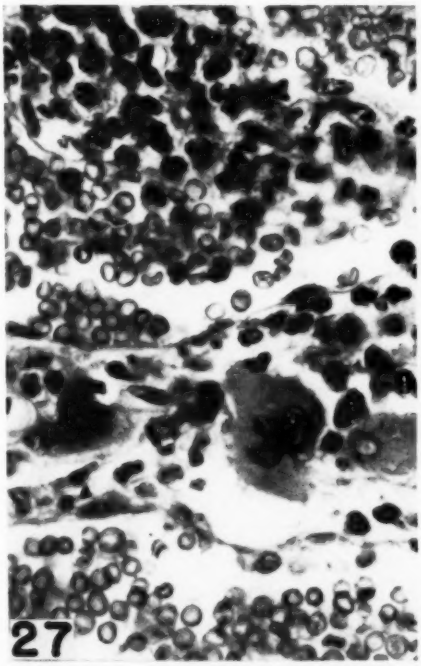
FIGURE 27.—High-power view showing medullary cords with granulocytes and megakaryocytes, and erythrocyte-filled spaces. × 650

FIGURE 28.—Low-power view showing many blood-filled cysts, and loss of normal structure. × 20

Types of reticuloendothelial hyperplasia in lymph nodes of the mouse.

FIGURE 29.—(C58 × C3Hf)F_1 male, 22 months of age. Marginal sinus crowded with elongated monocytic cells with intensely eosinophilic cytoplasm. × 260

FIGURE 30.—Medulla containing numerous round monocytic cells (reticulum cells) showing great variation in size. Cells of this type often appear actively phagocytic, and ingested erythrocytes and iron-positive debris can be recognized. Vacuolation of cytoplasm is observed in large cells shown here. × 260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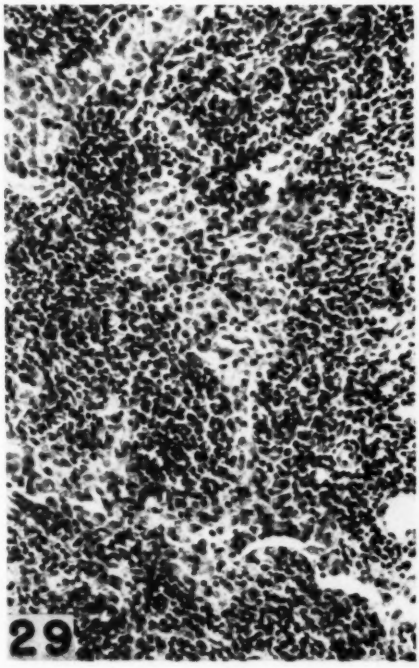
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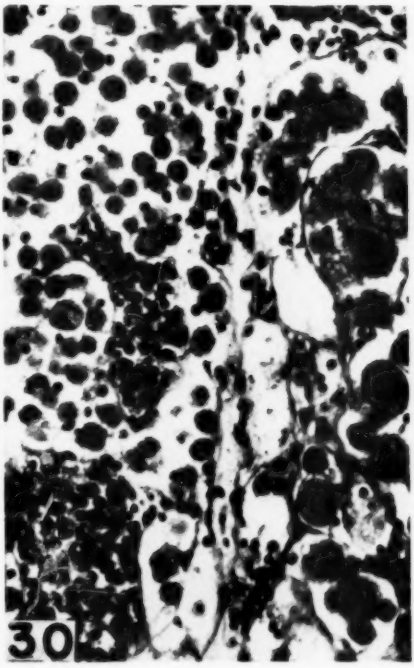
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PLATE 100

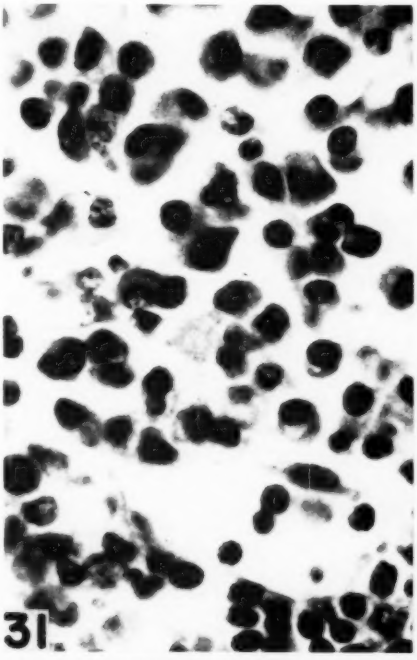
FIGURE 31.—(NH × AKR)F_1 male, 12 months old. Plasma-cell hyperplasia. Plasma cells may replace many lymphocytes in the lymph nodes of older mice. A mass of remaining lymphocytes is shown at lower left. × 210

FIGURE 32.—Hyperplasia of cells with Russell's bodies. × 880

Examples of granulomatous lesions in the mouse.

FIGURE 33.—(C57BL × DBA)F_1 female, 24 months old, painted with methylcholanthrene. Margin of a small abscess in a lymph node. Necrotic center appears at lower right, surrounded by monocytic cells, among which are Langhans-type giant cells. × 370

FIGURE 34.—Strain HR female, 13 months old, used as breeder. Unusual granulomatous lesion in periportal tissue of liver. Langhans-type giant cells are present, and the central cytoplasmic areas show early calcareous deposition. × 360



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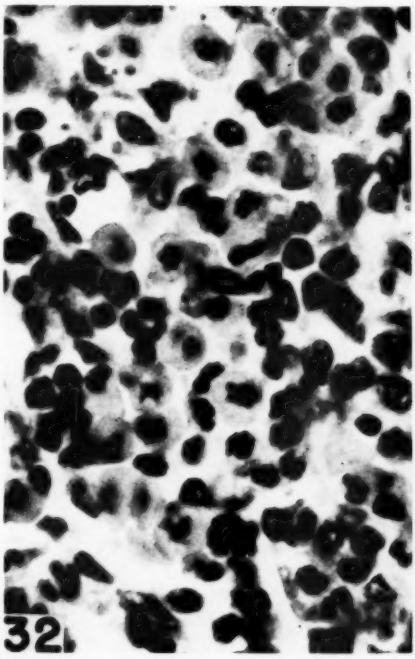

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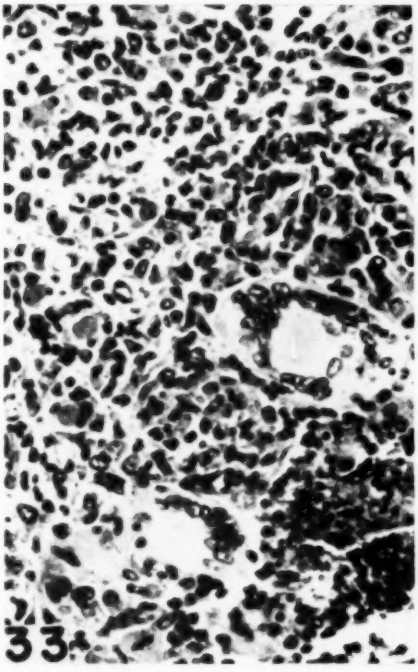

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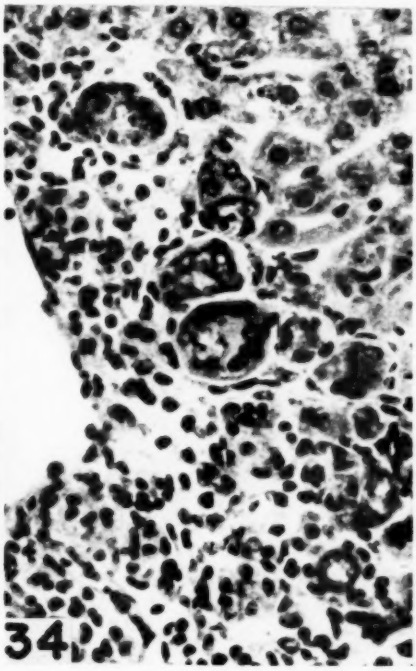

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PLATE 101

FIGURE 35.—Strain AKR male, 8 months old. Spontaneous lymphocytic neoplasm. Enormous enlargement of thymus, moderate generalized enlargement of lymph nodes and spleen. × 1

FIGURE 36.—Strain A male, 12 months old. Spontaneous lymphocytic neoplasm. Enormous enlargement of spleen. Thymus and lymph nodes normal. × 1½

FIGURE 37.—Strain C58 male, 10 months old. Lymphocytic neoplasm. Cells are larger than normal lymphocytes and mitotic figures are frequent. × 800

FIGURE 38.—Strain C58 female, 11 months old. Lymphocytic neoplasm. Leukemic cells are infiltrating skeletal muscle. × 400

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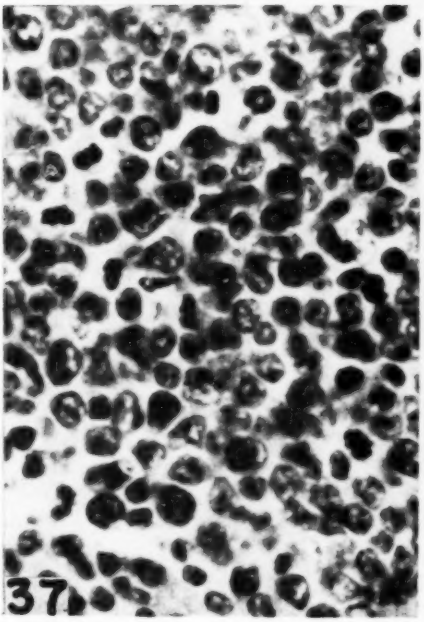
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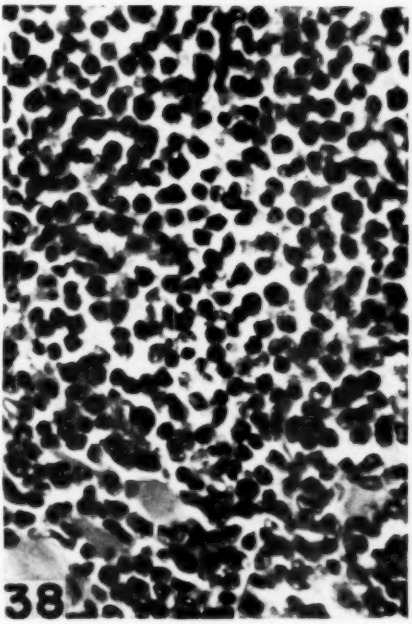
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PLATE 102

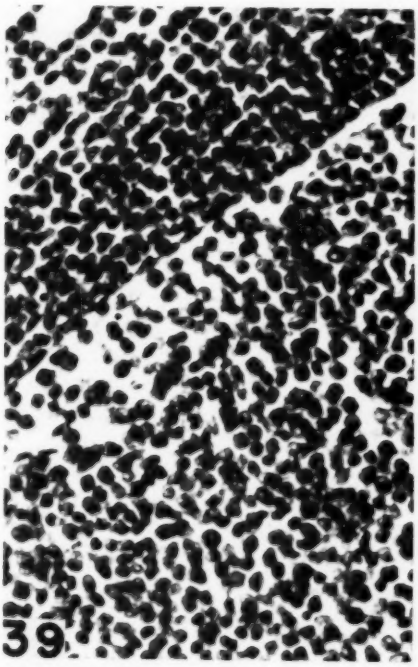
FIGURE 39.—(C3Hf × NB)F_1 male, 25 months old, painted with methylcholanthrene. Typical lymphocytic neoplasm. Margin of lymph node showing small lymphocytic cells diffusely scattered and replacing normal tissue, and spreading beyond the capsule. × 400

FIGURE 40.—(NH × AKR)F_1 female, 26 months old. Lymphocytic neoplasm in which cells are larger, with relatively more cytoplasm, and they appear to be clumped together. × 400

Examples of neoplastic lymphocytes in the liver, taken at same magnification (× 880) to show differences in size.

FIGURE 41.—Strain C58 female, 15 months old. Lymphocytic neoplasm composed of small cells diffusely scattered in sinusoids of liver.

FIGURE 42.—Strain AKR male, 10 months old. Neoplastic cells are considerably larger in size. Liver tissue shows fatty change.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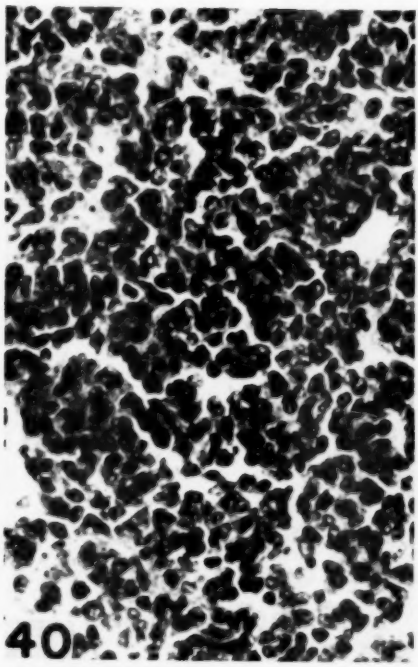
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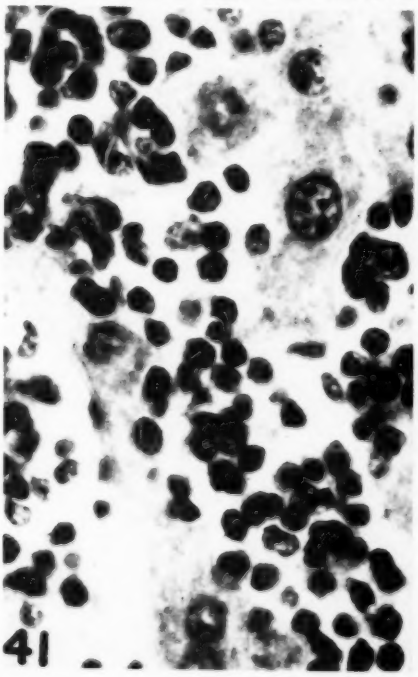
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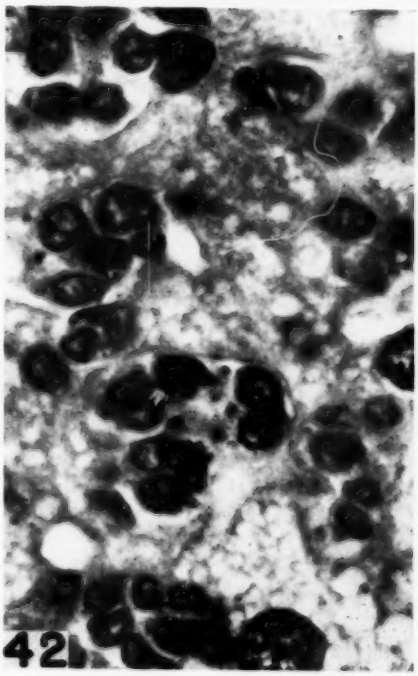
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PLATE 103

Extramedullary hematopoiesis.

FIGURE 43.—Extramedullary granulocytopoiesis in the adrenal gland. Much of cortex is replaced by developing granulocytes. × 200

FIGURE 44.—Strain BALB/c male, 4 months old, bearing a transplanted myoepithelioma. Section of pulmonary vessel and lung tissue. Note abundant granulocytes in lumen of vessel and infiltrating alveolar walls. × 520

Granulocytic leukemia.

FIGURE 45.—Ringform granulocytes, and less differentiated granulocytic cells infiltrating tissue of kidney. × 690

FIGURE 46.—Collection of undifferentiated granulocytes in a lymph node intermingled with many large mononuclear cells with peculiarly streaked eosinophilic cytoplasm. × 690

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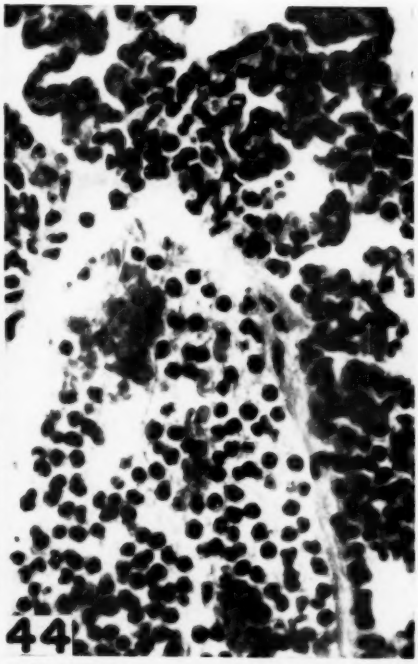
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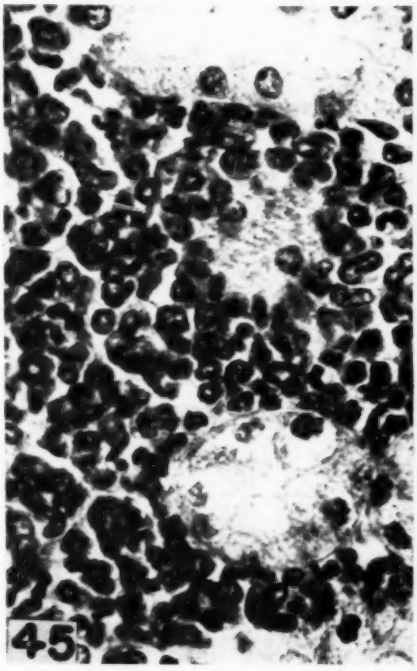
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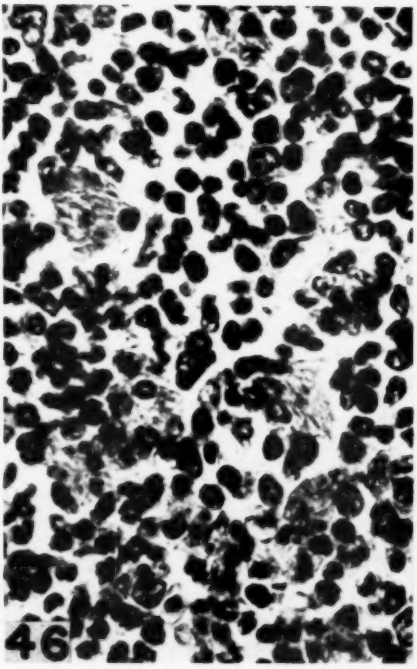
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PLATE 104

Reticulum-cell neoplasm, Type A (reticulum-cell sarcoma).

FIGURE 47.—(C57L × A)F_1 female mouse, 18 months old. Reticulum-cell sarcoma involving vagina, uterus, and ovaries. × 1½

FIGURE 48.—(C57L × A)F_1 mouse, transplanted with reticulum-cell sarcoma 6 months before. No tumor found at site of transplant but liver was widely infiltrated and enormously enlarged. × 1½

FIGURE 49.—(NB × C3Hf)F_1 male, 18 months old. Tumor in liver. Large fusiform cells around a cystic space in which mononuclear and multinuclear giant cells are seen. × 400

FIGURE 50.—(C3Hf × C58)F_1 female, 19 months old. Type of growth in which cells are spindle-shaped and resemble some fibrosarcomas. × 280

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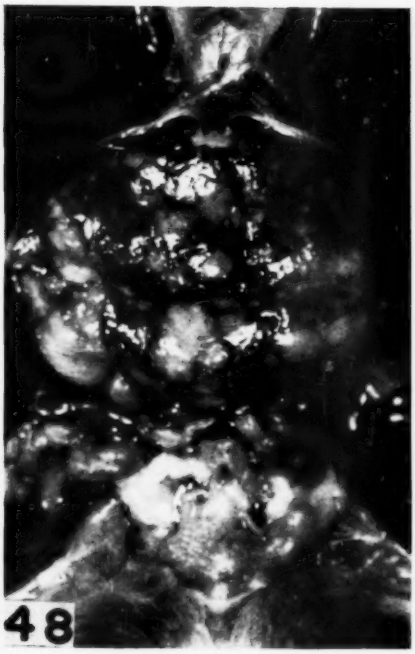
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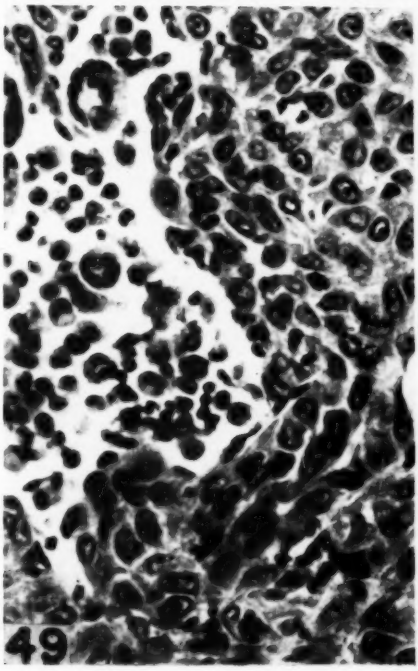
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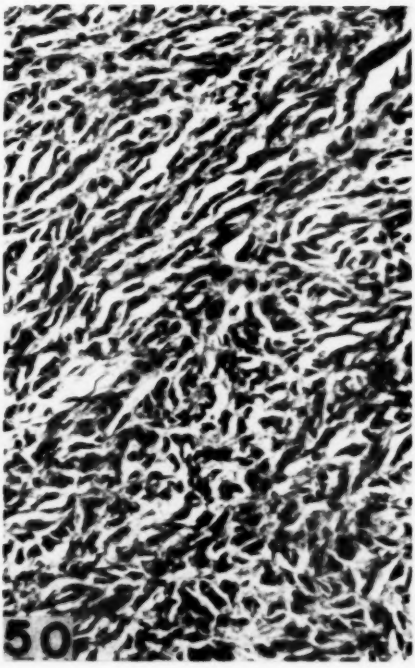
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PLATE 105

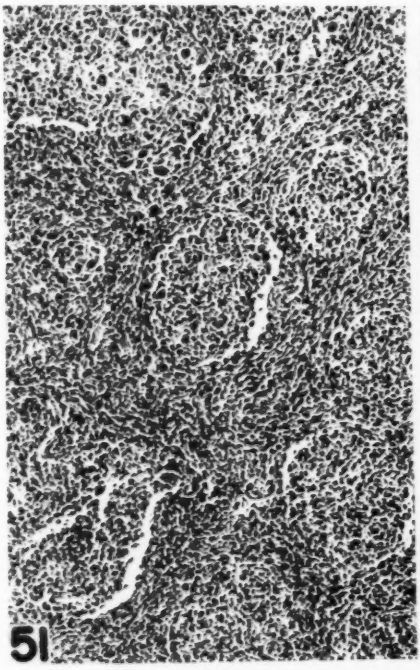
Reticulum-cell neoplasm, Type A.

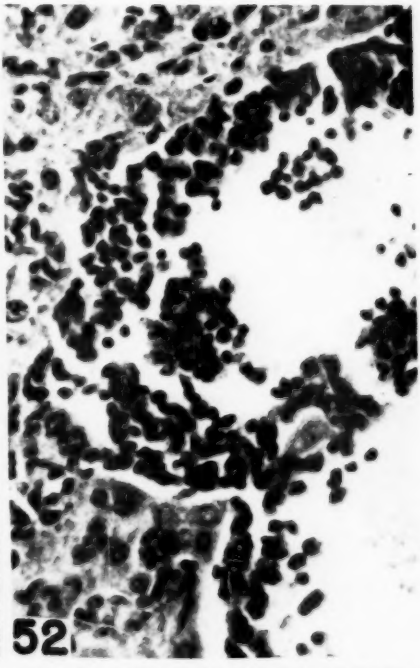
FIGURE 51.—Type of growth in which strands of fusiform cells surround and enclose nodules of rounded cells, some of which are multinucleate. × 110

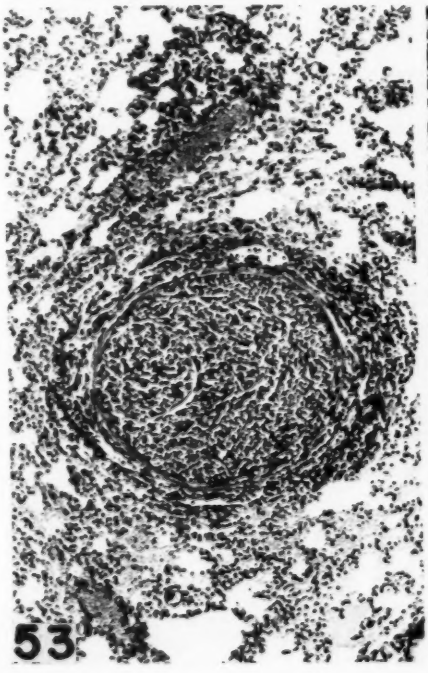
FIGURE 52.—Heavily pigmented (hemosiderin-containing) neoplastic cells in space within portal area of the liver. Evidence of phagocytosis of red-cell debris is frequently found in these neoplasms. × 370

FIGURE 53.—Metastasis of reticulum-cell sarcoma in lumen of a pulmonary vessel, and extending through the wall. Note resemblance to a fusiform fibrosarcoma. × 370

FIGURE 54.—Growth in liver replacing liver cords, and forming an angiomatous pattern. × 210


51


52


53

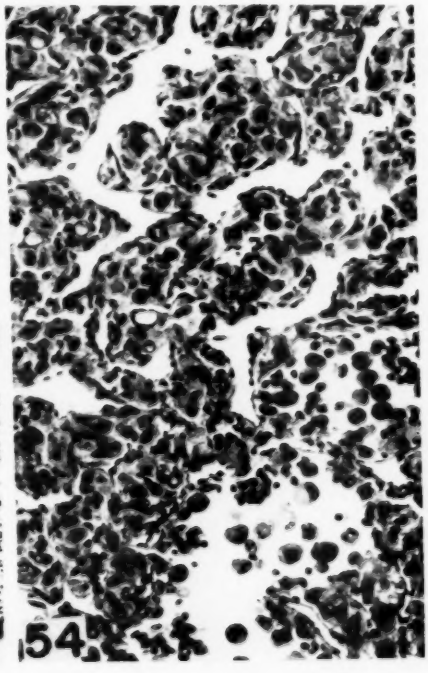

54

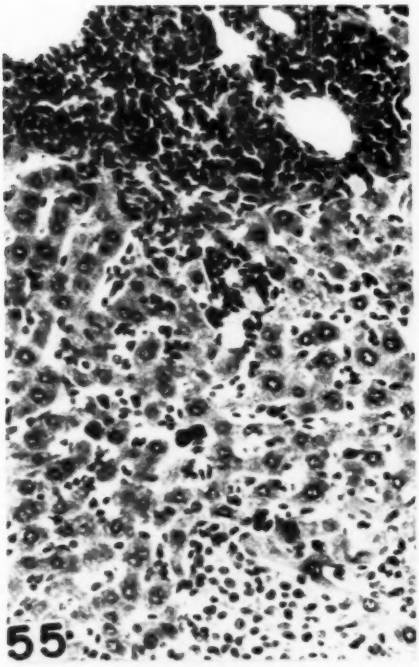
PLATE 106

FIGURE 55.—Liver. Reticulum-cell neoplasm, Type A (*bottom*) and area of granulocytopoiesis (*top*). × 210

FIGURE 56.—Liver. Reticulum-cell neoplasm, Type A (*bottom*) and lymphocytic neoplasm (*top*) in mouse with transplanted reticulum-cell tumor in which lymphocytic neoplasm apparently developed spontaneously. × 140

FIGURE 57.—Strain C57L male, 24 months old. Reticulum-cell neoplasm, Type B. Extreme enlargement of the mesenteric lymph node. Spleen is moderately enlarged and white nodules can be seen in it. × 1

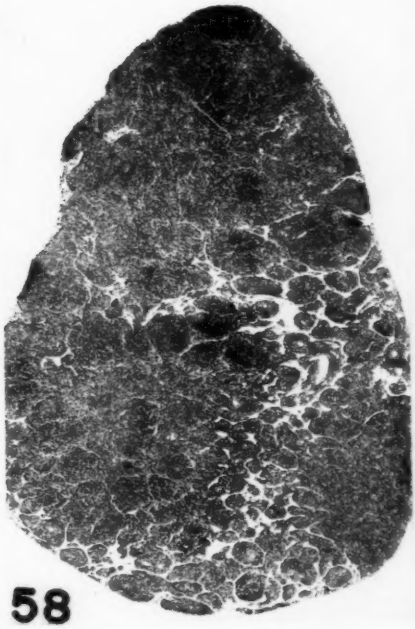
FIGURE 58.—(NH × AKR)F_1 mouse, 24 months old. Lymph node showing Type B lesion. Low-power view of entire node shows replacement by abnormal tissue and loss of normal architecture. Cortex and medulla are replaced by pleomorphic Hodgkin's-like tissue, which is confined within the capsule of the node. Medullary sinuses are still seen. × 18



55

56

57



58

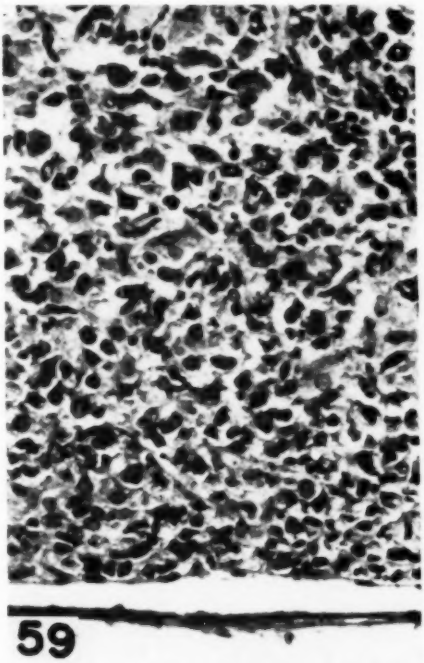
PLATE 107

FIGURE 59.—Lymph node which is site of the Type B neoplasm, showing that the capsule is still intact. × 300

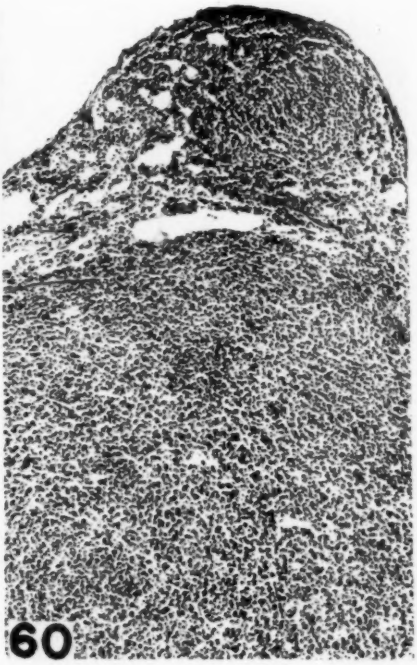
FIGURE 60.—Reticulum-cell neoplasm, Type B, in the spleen. Note involvement of centers of splenic nodule. × 75

FIGURE 61.—Reticulum-cell neoplasm, Type B (Hodgkin's-like lesion) originating in a Peyer's patch. Note variety of types of cells. A background of pale mononuclear cells sprinkled over with many lymphocytes, plasma cells, and a few granulocytes. Eosinophils are rarely found. (This lesion was successfully transplanted through 2 generations.) × 520

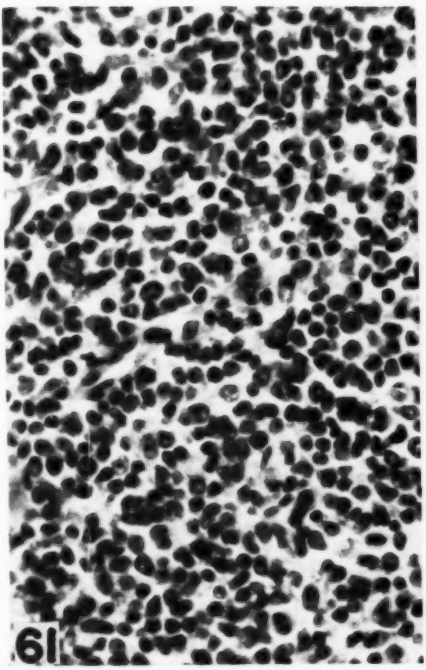
FIGURE 62.—High-power view of Type B neoplasm in spleen showing large, pale cells, nuclear pyknosis and pleomorphism of cell forms. × 900



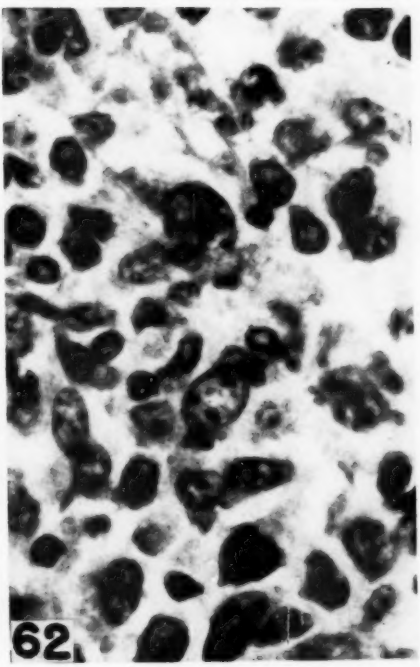
59



60



61



62

PLATE 108

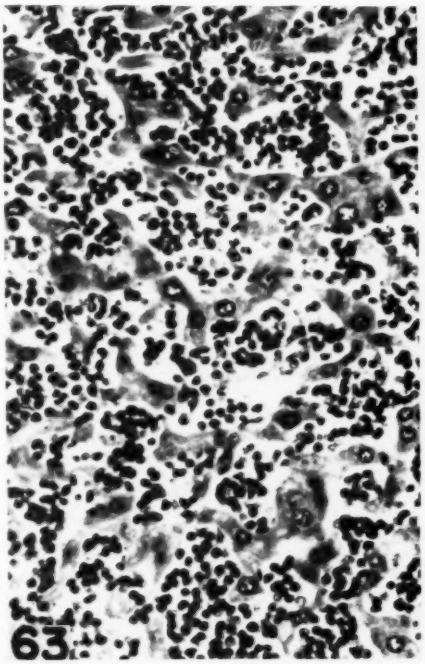
To show morphologic differences in various types of reticular neoplasms when growing in the liver. × *280*

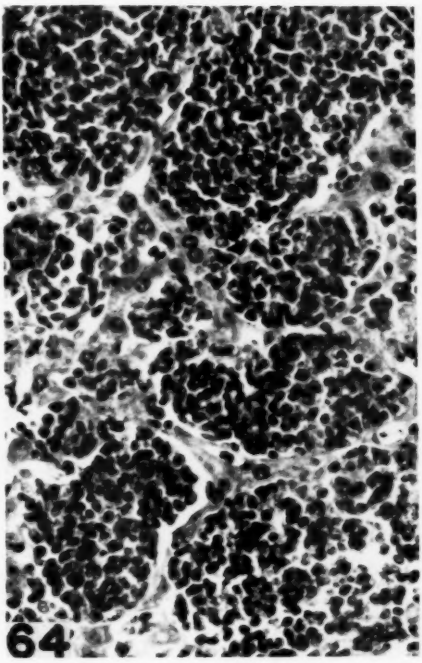
FIGURE 63.—(C58 × C3Hf)F_1 female, 13 months old. Lymphocytic neoplasm composed of small cells with round, heavily stained nuclei and scant cytoplasm. The cells are generally dispersed through sinusoids of liver. This is a "typical," easily classified type.

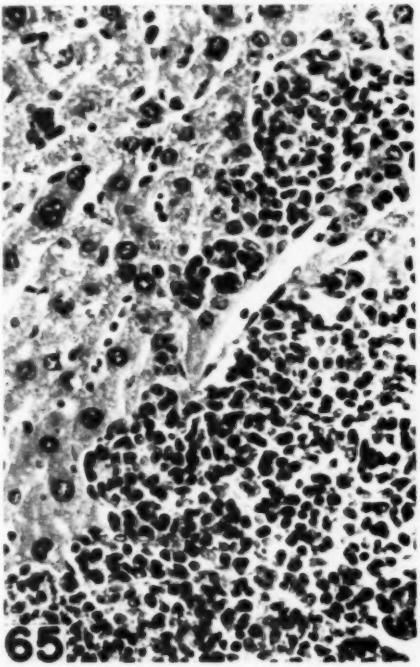
FIGURE 64.—(C3Hf × C58)F_1 male, 28 months old. Lymphocytic leukemia, composed of larger cells with round, oval or slightly indented nuclei, and more abundant cytoplasm. The cells tend to be clumped together, and some areas in these neoplasms may even suggest a glandular pattern. This type is difficult to classify since it is borderline with reticulum-cell neoplasm, Type B. Criteria relied upon are the general uniformity of the cells, and the resemblance to large lymphocytes.

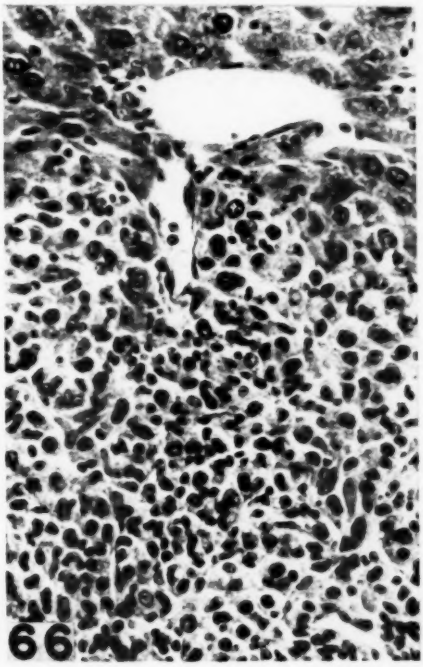
FIGURE 65.—(C3Hf × C58)F_1 male, 35 months old. Reticulum-cell neoplasm, Type B, composed of large, pale monocytic cells with an admixture of other cell forms, principally lymphocytes. Abnormal tissue is found in large masses, clearly separated from liver tissue.

FIGURE 66.—(C3Hf × C58)F_1 male, 19 months old. Reticulum-cell neoplasm, Type A, composed of large polymorphic cells with abundant cytoplasm. Neoplastic tissue appears to infiltrate normal liver.









289972—54——20

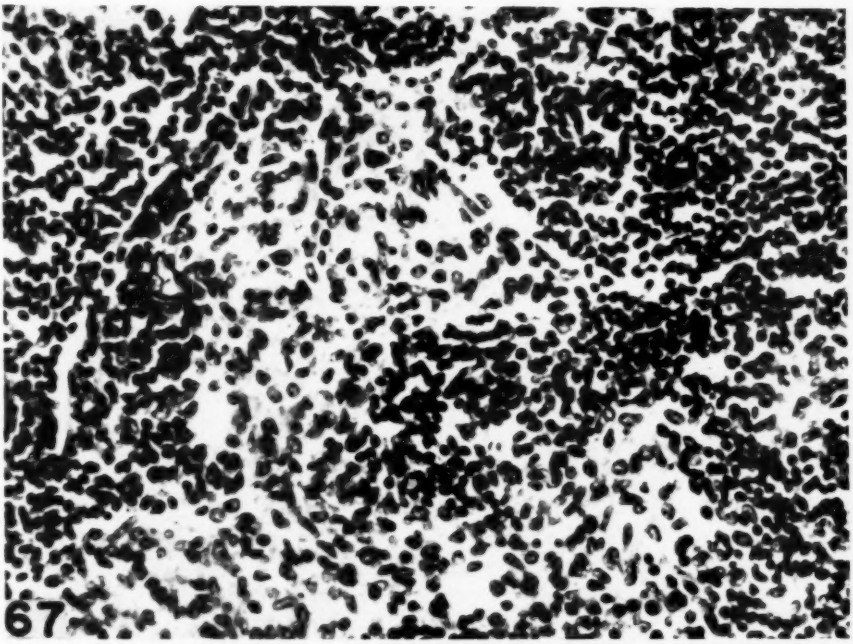
PLATE 109

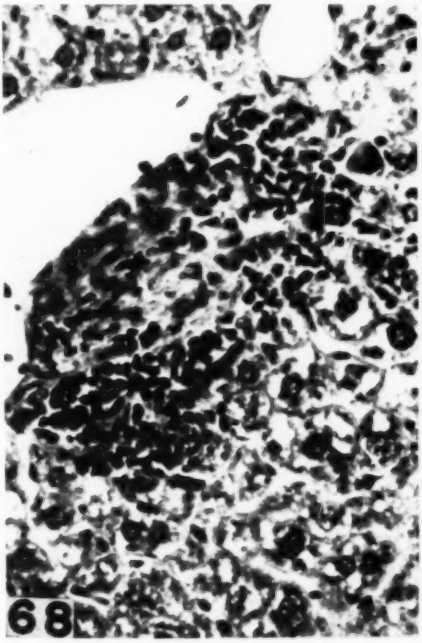
Reticulum-cell neoplasm, Type C.

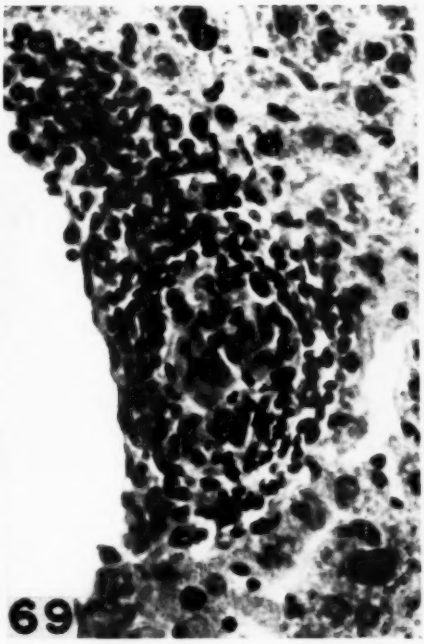
FIGURE 67.—Lesion in a lymph node. Node was greatly increased in bulk, and microscopically tissue was replaced by sheets of pale-staining, large, fusiform cells bordered by masses of small lymphocytes. × 360

FIGURE 68.—High-power view of the lesion in liver in same mouse. Multiple lesions of this type appeared in the periportal areas. A central mass of large, pale, fusiform mononuclear cells was surrounded by a mantle of small lymphocytes. × 360

FIGURE 69.—Strain HR male, 23 months old, painted with ether as control for methylcholanthrene painting. Lesion of undetermined type in liver consisting of central large, heavily pigmented cells surrounded by small lymphocytes. Multiple lesions of this type are often found in old mice in which the incidence of other lesions of the reticular system is high. × 400


67


68


69

Rare neoplasms of reticular tissues

PLATE 110

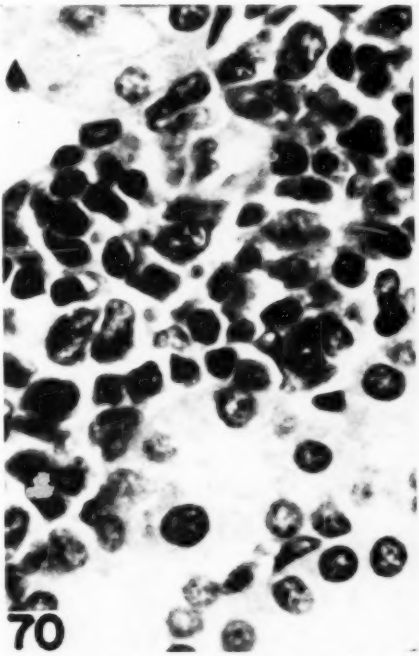
FIGURE 70.—Plasmacytic neoplasm showing an infiltration of the kidney by neoplastic plasma cells in various stages of differentiation. × 900

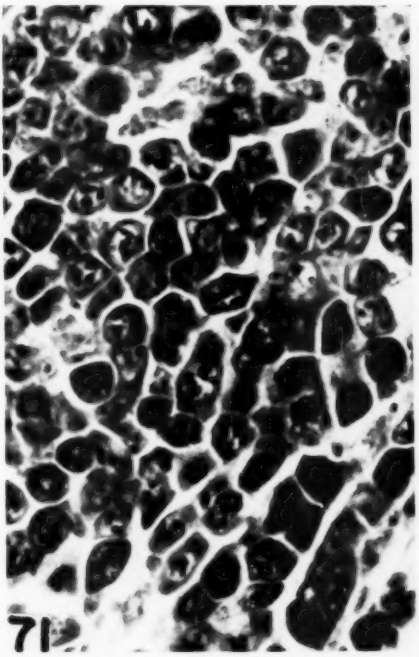
FIGURE 71.—Strain C3Hf mouse, 22 months old. Plasma-cell neoplasm developing in submucosal tissue at lower end of ileum, and involving mesenteric lymph node. Cells are large with prominent nucleus and nucleoli. Nuclei eccentric, cytoplasm a violet color, and often a clear area near nucleus. Giemsa. × 690

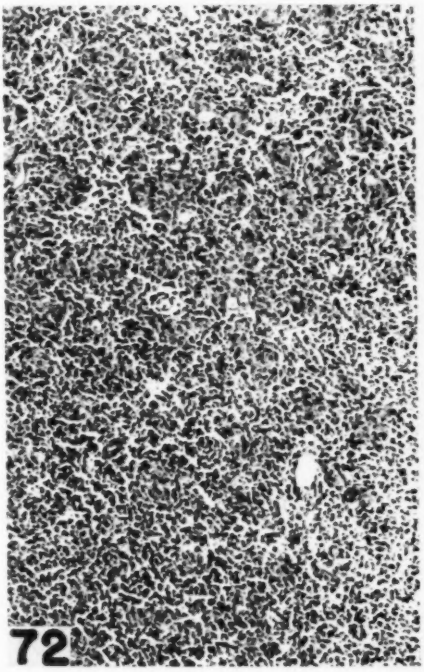
Strain 1194 male, 8 months old. Breeder. Thymoma.

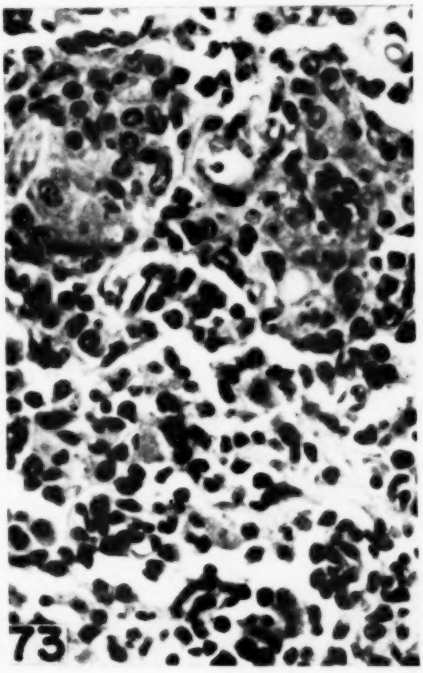
FIGURE 72.—A low-power view showing proliferation of thymus reticulum intermingled with lymphocytes. Thymus was considerably increased in bulk, and microscopically replaced throughout by this type of tissue. × 100

FIGURE 73.—High-power view to show whorl-like masses of reticulum cells, resembling Hassall's corpuscles. × 385


70


71


72


73

Rare neoplasms of reticular tissues

PLATE 111

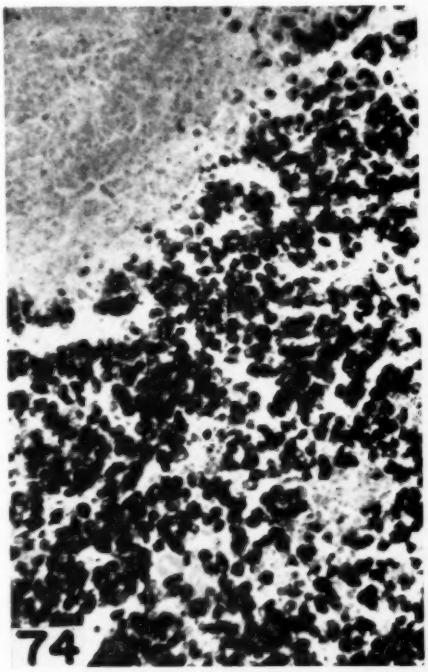
FIGURE 74.—Strain C58 male, 9 months old. Mast-cell neoplasm. Cells are large and round and contain metachromatic granules stained with toluidine blue. The cells are crowded in the red pulp of the spleen, while the splenic nodules are free. × 210

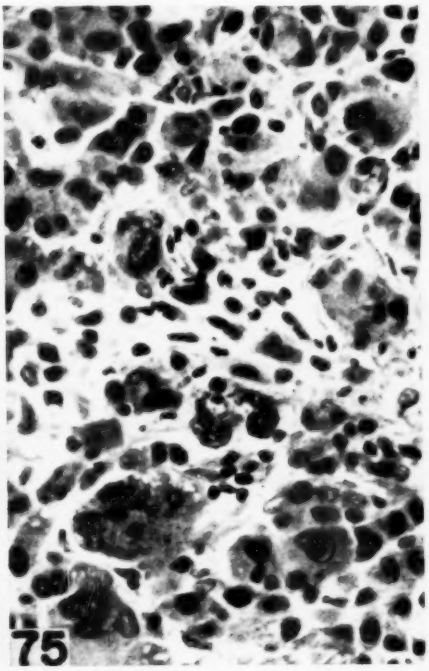
FIGURE 75.—Strain HR male, 14 months old. Unclassified neoplasm probably of reticulum-cell type containing many multinucleate cells of giant size. A few other tumors of this type have been observed. × 370

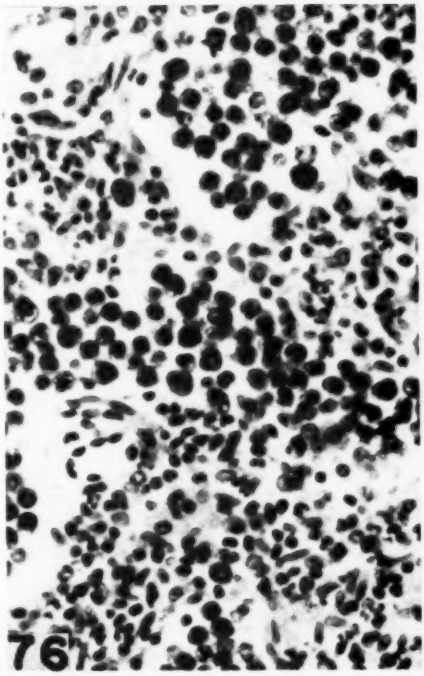
(C57L × A)F_1 female, 20 months old. Received 50 r whole-body irradiation at birth. Unclassified neoplasm.

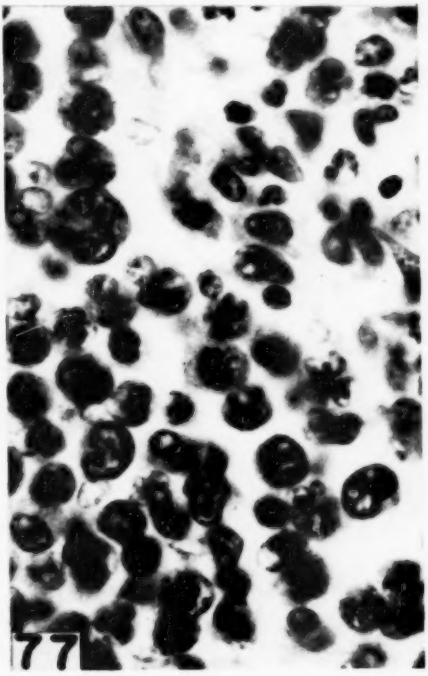
FIGURE 76.—A low-power view of diffuse tumor cells in the sinusoids of a lymph node. Active granulocytopoiesis is seen in the medullary cords. × 360

FIGURE 77.—Details of neoplastic cells. Nuclei are frequently indented or multilobed and vary greatly in size. This neoplasm is unlike any commonly seen in the mouse. × 880


74


75


76


77

INDEX OF AUTHORS

Volume 14

Journal of the National Cancer Institute

Page numbers in italics indicate senior authorship
(Page numbers in parentheses refer to discussions)

Adamson, Dorothy M., 941
Algire, Glenn H., (687), (719), *865*, *879*
Anderson, Belle F., 879
Andervont, Howard B., *317*, *329*

Barrett, Morris K., 375, *381*
Barnett, Russell J., *769*
Bassett, C. Andrew, (741), (743)
Baxter, Hamilton, (741)
Beard, Dorothy, 1011, 1027, 1039, 1055, 1067
Beard, J. W., 1011, 1027, 1039, 1055, 1067
Beck, Lyle V., *1223*
Belkin, Morris, *607*
Bennett, James G., 105
Binhammer, Robert T., 149
Black, Maurice M., *1147*
Borges, P. R. F., 481
Braker, William P., 513
Brecher, G., *159*
Brown, Mary B., 303
Bryant, Jay C., *189*, 513, 853, 1159
Buchanan, Talmadge, (743)
Burgoyne, Frederick H., 805
Burmester, B. R., 1067

Chase, Herman B., 341
Colsky, Jacob, 1257
Congdon, Charles C., 291, 571, 955
Conway, Herbert, (685), (688)
Cronkite, E. P., 159
Cunningham, A. W. B., 149

Damron, Charlotte M., 93, *279*
Deringer, Margaret K., *127*, *375*, 381
Duncan, Helen M., 395
Dunn, Thelma B., 317, 329, 493, *1281*
Dyer, Helen M., *93*, 279

Earle, Wilton R., 189, 513, 655, *841*, *853*, *1159*, 1173
Eckert, Edward A., 1011, 1027, *1039*, *1055*, *1067*
Eichwald, E. J., *705*
Eisen, Arthur Z., *341*
Ekstein, Daniel M., 45
Ellerbrook, Lester D., 395
Ermala, Pentti, 141
Evans, Virginia J., 513, 655, (741), 1159, 1173

Fekete, Elizabeth, 485
Finerty, John C., *149*
Fisher, Edwin R., *355*
Fitzgerald, Dorothea B., 607
Flint, J. S., 439
Fong, Conrad T. O., 395

Gabrielson, F., 457
Gaillard, P. J., (683)
Gellhorn, Alfred, *935*
Gillespie, Ruth Joyce, 1223
Goldfeder, Anna, (720)
Goldin, Abraham, *1247*
Greenspan, Ezra M., *1257*

Hansen, Walter H., 381
Hare, William V., 105
Hartwell, J. L., 365, 645, 967
Hauschka, Theodore S., 1, *723*
Hertz, Roy, 1173
Heston, W. E., 127, *131*, 375, (701)
Heyndrickx, A., 1233
Highhouse, Frederick, 841
Hill, Elizabeth L., 819
Hirschberg, Erich, 935
Hollcroft, Joanne W., *527*

Journal of the National Cancer Institute, Vol. 14, No. 6, June 1954
289972—54——21

Hsu, T. C., *905*
Hume, David M., (685)

Ikawa, Miyoshi, *1195*

Kahler, J. S., 365
Kaliss, Nathan, (717)
Kaplan, Henry S., *303*
Kells, Alice, 935
Kelly, Margaret G., *967*
Kertész, Denis, *1081*, *1093*
Klein, George, *229*
Klein, Michael, *77*, *83*, *1137*
Kline, I., 365, 449, 645
Koepfli, J. B., 1195
Kolthoff, I. M., *1233*
Koprowska, Irena, *627*
Koprowski, Hilary, 627

Laszlo, Daniel, 45
Leadbetter, Patricia A., 513
Legallais, Frances Y., 879
Leiter, J., *177*, *365*, *449*, 585, *645*
Levan, Albert, *1*
Lewin, Ruth, *45*
Lippincott, Stuart W., *395*
Longmire, W. P., Jr., *669*
Lorenz, Egon, *291*, *955*
Lowe, Jere W., 423

Main, Joan M., 537, 895, 1277
Malmgren, Harry, *1119*
Malmgren, R. A., *411*
Matthews, Marion, 527
Medawar, P. B., (703), (717), (767)
Merwin, Ruth M., (719), *819*
Miller, F. R., 439
Mommaerts, E. B., *1011*, 1027, 1039
Montagna, William, 341
Morren, Loes, 1233
Morris, Harold P., 93, *493*, 593
Mudd, Seeley G., 1195
Mulay, Ambadas S., *571*, 805

Neptune, Wilford B., (684)
Niemann, Carl, 1195

Paradis, Anita D., 177, 585
Passonneau, Janet V., 941
Pate, James W., (685)
Paull, Janice, 303
Pearson, Bjarne, 1123
Peers, James H., 159
Peppers, Ellison V., 189
Prehn, Richmond T., *537*, (701), *895*, *1277*

Rathgeb, Paul, *1099*, *1109*
Reeve, Robert H., 1039
Révész, László, 229
Rhees, Mark C., 395
Rhoads, Jonathan E., (683)
Richardson, Flavia L., *1123*
Rogers, Blair O., (684), (718)
Russell, Elizabeth, 485

Sandstead, Harold R., *423*
Sanford, Katherine K., *513*
Saxén, Erkki A., 411, *547*
Schilling, Edward L., 853, 1159
Schneider, Martin, 149
Schneiderman, Marvin, 895
Schoenbach, Emanuel B., 1257
Scow, R. O., 593
Seligman, Arnold M., 769
Setälä, Kai, *141*
Shannon, John E., Jr., 655
Sharp, D. G., 1011, *1027*, 1039
Shear, M. J., 365, 645, 1257
Siurala, Max, 141
Smith, Priscilla, 457, 485
Snell, George D., *457*, *481*, *485*, *691*
Speer, Francis D., 1147
Steiner, Paul E., *1205*
Stern, Kurt G., 45
Stewart, Harold L., *105*
Stewart, Sarah E., *1187*
Stricks, W., 1233
Sylvén, Bengt, 1099, 1109
Symeonidis, Alexander, *805*

Tahmisian, Theodore N., *941*
Thornton, Helen, 395
Toolan, Helene Wallace, *745*
Tullner, William W., 1173
Turner, D. L., *439*

Uphoff, Delta, 291

Wagner, Billie P., 493
Waltz, Helen K., *1173*
Waravdekar, V. S., 177, *585*
Waters, Nelson F., 1067
Waxler, Samuel H., *1253*
Westfall, Benton B., *655*
Wilson, J. Walter, *57*, *65*, (743)
Winnick, Ruth E., *519*
Winnick, Theodore, 519
Woidowsky, Leonard, 45
Wollman, S. H., *593*
Woolley, George W., (703)

INDEX OF SUBJECTS

Volume 14

Journal of the National Cancer Institute

2-Acetylaminofluorene-N^{15}, recovery from rats, 93, 279
Acetylpodophyllotoxin-ω-pyridinium chloride, effect on tumor enzymes, 585
ACTH, resistance to grafts and, 745
  skin grafts and, 684, 718
Adaptation, of leukemia to various strains, 1187
  of tumors, specificity, 381, 723
Adenine, effect on 8-azaguanine carcinostasis in rabbits, 935
Adenocarcinomas, induced in stomach by methylcholanthrene, 105
  intestinal, X-ray-induced in protected rats, 159
  mammary C3HBA, response to various X-ray treatments, 527
    specificity of induced adaptation, 381
    See also Carcinomas, mammary; Mammary tumors
Adenosine, effect on 8-azaguanine carcinostasis in rabbits, 935
  protective against 6-mercaptopurine toxicity, 1247
Adenosine triphosphatase:
  relation to virus particles, 1011
    electrophoresis and sedimentation, 1027
    infectivity and, 1039
Adenylic acid, effect on 8-azaguanine carcinostasis in rabbits, 935
  protective against 6-mercaptopurine toxicity, 1247
Adrenal cortex:
  hormones, effects on reticular tissues, 1307
  tumors, genetic susceptibility to, 703
Adrenal glands, effect on liver tumorigenesis, 805
  response to gonadotrophin, B_6 deficiency, restricted diet, 493
Adrenocorticotrophic hormone. *See* ACTH
Age, effects on:
  mammary tumor distribution, 895
  ovarian tumors, 77
  reticular tissues, 1311
  tumorigenesis, 547
Agglutinins, and immunity to tumor grafts, 705
Albumin, in normal and cancer sera—nitrogen, sulfhydryl, disulfide in, 1233
Alkaline phosphatase, during mammary tumorigenesis, 1123
Amino acids, in culture media, 513
    utilization, 519
  in Shear's polysaccharide, 1109
p-Aminopropriophenone, protects rats against X ray, 159
Aminopterin, effect on *in vitro* dehydrogenase of human cancer, 1147
Anaerobiosis, grasshopper embryos in, 941
Anemia, from pyridoxine deficiency, 493
Announcements, 227, 228, 455, 456, 1203
Antibodies, and immunity to grafts, 705
Antigens, in rabbit tumors and kidneys, comparison, 395
Antigen II, and histocompatibility-2 locus, 691
Antimonials, effect on tumor cytochrome oxidase, 177
Arsenicals, effect on tumor cytochrome oxidase, 177
  lethality in tumor-bearing mice, 1223

Ascites tumors:
effect of Mengo virus on, 627
of radiolanthanum on, 45
endomitosis in, 1
from solid tumors, 723
growth of (quantitative study), 229
human, chromosomes *in vitro*, 905
8-Azaguanine, reversal of carcinostatic action in rabbits, 935
Azo dyes. *See* names of compounds

Bacteria. *See Escherichia coli; Serratia marcescens*
Bentonite, in diet, hepatomas induced, 65
nutritional deficiency caused, 57
Bile, acetylaminofluorene recovery from, 93, 279
Biopsy, gastric, instrument for, 141
Biphenyls, effect on Sarcoma 37, 365
Blood, of mice, methods for examining, 1285
review of literature, 1283
Blood cells in mice, 1289
effect of pyridoxine deficiency on, 493
See also Reticular tissue
Blood-forming organs. *See* Hematopoietic tissue
Blood plasma:
of leukemia patients, myelokentric and lymphokentric acids in, 439
virus particles in, relation to enzyme activity, 1011, 1027
infectivity and, 1039
Blood pressure (peripheral), effects of podophyllin on, 879
measurement of, 865
Blood serum:
fraction, in culture media, 513
normal and in cancer patients—nitrogen, sulfhydryl, disulfide in, 1233
of leukemia patients, myelokentric and lymphokentric acids in, 439
See also Complement fixation
Blood vessels, clinical grafting, 669
disulfides and sulfhydryls in, 769
See also Vascularization
Bone, clinical transplantation, 669
injections of, protective against X rays, 955
Bone marrow in mice, 1281
depression of, and lymphoma induction by X ray, 291
formation in bone transplants, 955
injections of, and X-ray-induced lymphoma incidence, 303
thymic recovery and, 303
Bone marrow—*Continued*
methods for examining, 1287
morphology, 1300
protected from X ray by spleen shielding, 291
See also Reticular tissue
Brown-Pearce carcinoma. *See under* Carcinomas

Cancer, emphasis in research on, 1205
See also Tumors
Carbohydrate, in Shear's polysaccharide, 1099
composition, 1109
Carcinogenesis. *See* Tumorigenesis
Carcinogens. *See* Acetylaminofluorene; Dibenzanthracene; *p*-Dimethylaminoazobenzene; *p*-Dimethylaminobenzene-1-azo-2-naphthalene; Estrogens; Methylcholanthrene; Mustards; Urethan; X radiation
Carcinomas:
ascites, endomitosis in, 1
Brown-Pearce, complement fixation in rabbits bearing, 395
reversal of 8-azaguanine inhibition, 935
human, chromosomes *in vitro*, 905
dehydrogenase affected by chemotherapy agents, 1147
response to α-peltatin, 1257
mammary 15091a, genetics of transplantation, 457
use to classify *H-2* alleles, 457, 481
See also Adenocarcinomas, mammary; Mammary tumors
squamous-cell, induced by fetal-tissue grafts, 1137
See also Adenocarcinomas; Ehrlich ascites tumor
Cartilage, clinical transplantation, 669
Cell proliferation:
effect of amino acids on, 513
of rotation velocity on, 853
in filtered embryo extract, 189
in fluid-suspension cultures, 1159
utilization of amino acids, 519
See also Culture media; Tissue culture
Cells:
ascites tumor, effects of Mengo virus on, 627
endomitosis in, 1
rates of multiplication, 229
blood. *See* Blood cells
effects of podophyllin on, 967
of hydatid-mole cultures, 1173

Cells—*Continued*
of mouse leukemia, studies on, 1334
of reticular tissues in mice, 1281
hyperplasia of, 1313
neoplasms of, 1321
Shope fibroma, inclusions in, 355
See also names of cells
Centrifugation. *See* Ultracentrifugation
Cheek, mucosa, leukoedema of, 423
Chemotherapy. *See* Tumor-damaging agents
Chick embryos. *See under* Embryos
Choline deficiency, and effects of bentonite feeding, 57, 65
and hepatoma induction, 65
Chromosomes, in ascites tumors, 1
in human tumors *in vitro*, 905
ploidy, effect on tumor growth, 723
Claw (rat), disulfides and sulfhydryls in, 769
Colchicine and derivatives:
effects on Sarcoma 37, 365, 645
on tumor cytochrome oxidase, 177
on tumor respiration and enzyme, 585
Complement fixation, in tumor-bearing rabbits, 395
Condyloma acuminatum, effects of podophyllin on, 967
Copper, relation to human melanoma phenoloxidase, 1081
Cornea, clinical transplantation, 669
Cortisone, resistance to grafts and, 745
Croton oil, effects on skin tumorigenesis, 83
Culture media:
amino acids as supplements, 513
amino acid uptake from, 519
glucose uptake from, 655
filtration of embryo extract, 189
serum, embryo-extract fractions, 513
See also Cell proliferation; Tissue culture
Cytochrome c, tumor, effect of podophyllotoxin derivative on, 585
Cytochrome oxidase, tumor, effects of tumor-damaging agents on, 177, 585

Dehydrogenase, of human cancer, chemotherapy agents and, 1147
succinic, effect of podophyllotoxin derivative on, 585
4′-Demethylpodophyllotoxin, 986
Desoxycorticosterone acetate, effect on liver tumorigenesis, 805
1,2,5,6-Dibenzanthracene, pulmonary tumors induced by, 127
Diet (restricted), and rate of tumorigenesis, 1253
effects on gonadotrophin-injected, B_6-deficient mice, 493
Digestive system, disulfides and sulfhydryls in, 769
p-Dimethylaminoazobenzene, liver lesions induced by, adrenal glands and, 805
p-Dimethylaminobenzene-1-azo-2-naphthalene, liver tumors in rats fed, 571
Diphenylpropylamines, effect on Sarcoma 37, 645
Diphosphopyridine nucleotide, protective against 6-mercaptopurine, 1247
Disulfides, in normal and cancer sera, 1233
protein-bound, histochemistry, 769
Dopa-oxidase, in human melanomas, 1081
oxygen affinity compared with potato enzyme, 1093
Dose-responses:
of tumors to X-ray treatments, 527
to avian leukosis virus, strain differences, 1067
titration, 1055
Dyes, effect on tumor transplantation, 745

Edema, mucosal. *See* Leukoedema
Ehrlich ascites tumor:
effect of Mengo virus on, 627
of radiolanthanum on, 45
growth of (quantitative study), 229
Electrophoresis, of Shear's polysaccharide, 1119
of virus particles and enzyme, 1027
and infectivity, 1039
Embryo extract. *See* Culture media
Embryos, chick, amino acid utilization by heart cultures, 519
grasshopper, relation of metabolic rate to X-ray damage, 941
mouse, growth rate compared with ascites tumors, 229
Encephalitis, Mengo. *See under* Virus
Endocrine glands, disulfides and sulfhydryls in, 769
Endomitosis, in ascites tumors, 1
Enzymes, effects of tumor-damaging agents on, 585
phenol-oxidizing, in human melanomas, 1081
oxygen affinity compared with potato enzyme, 1093
See also Adenosine triphosphatase; Alkaline phosphatase; Cytochrome oxidase; Dehydrogenase; Hyaluronidase

Eosinophils, in mice, 1293
Epithelium, disulfides and sulfhydryls in, 769
effects of podophyllin on, 967
tumors, X-ray-induced in rats, 159
Erythrocytes, in mice, 1289
Erythromyeloblastic leukosis. *See under* Virus
Escherichia coli, tumor-hemorrhagic agent from, action of chemicals on, 1195
Estrogens, lymphocytic neoplasms induced in mice by, 1329 ff.
See also Stilbestrol
Etiology, cancer, problem for research, 1205
Excerpta Medica, section on cancer (announcement), 455

Fascia, clinical transplantation, 669
Feather (chicken), disulfides and sulfhydryls in, 769
Feces (rat), recovery of acetylaminofluorene from, 93
measurement, 279
Fibroma, Shope, cell inclusions, 355
Fibrosarcomas, in liver and spleen, induced by fetal-tissue grafts, 1137
Filtration, of embryo extract for culture media, 189
Fluctuation test, 723
Fluorenes, effect on Sarcoma 37, 365
Fractionation, of Shear's polysaccharide, 1099

3-Galactosidylquercetin, 986
Gall bladder, effects of podophyllin on, 967
Gastrointestinal tract:
adenocarcinomas, X-ray-induced in protected rats, 159
effects of α-peltatin on, 1257
of podophyllin on, 967
See also Stomach
Genes, adaptation of transplanted tumors and, 381
histocompatibility, isogenic resistant lines and, 691
tumor transplantation and, 485, 691
See also Histocompatibility-2 locus
linkage, test for *H-2* alleles, 457, 481, 691
Genotype, influence on mammary tumors: distribution, 895
incidence in hybrids, 317
Globulin, in normal and cancer sera—nitrogen, sulfhydryl, disulfide in, 1233
Glucan, in Shear's polysaccharide, 1099
properties, 1109
Glucose, uptake from culture medium, 655
Glutathione, protects against X-ray, 159
Glycogen, measured in cultures of 2 cell strains, 655
Gold thioglucose, tumorigenesis in mice given, 1253
Gonadotrophin, effects on B_6-deficient, diet-restricted mice, 493
secretion in hydatid-mole cultures, 1173
Grafts, acquired immunity to, 705
mobile resistance factor, 537, 1277
clinical (homostatic, homovital), 669
fetal tissues, tumors induced by, 1137
initial host reaction to, 687
lung, urethan carcinogenesis in, 411
methods of conditioning, 723
ovarian, tumorigenesis in, in old mice, 77
tumor, adaptation induced in, 723
specificity of, 381
vascularization, 687, 719
and host resistance, 819
See also Transplantation
Granulocytes, in mice, 1292
Granulocytic leukemia in mice, 1346
compared with extramedullary hematopoiesis, 1343
Granulosa-cell tumors. *See* Ovaries, tumors of
Grasshoppers. *See under* Embryos
Growth, and problem of cancer research, 1205
inhibition in mice fed bentonite, 57
of ascites tumors, measurements, 229
of transplanted tumors. *See* Transplantation, tumor
See also Cell proliferation
"Growth factors," effects on tumor grafts, 745
Guanine, aided 8-azaguanine carcinostasis in rabbits, 935
Guanosine, protective against 6-mercaptopurine toxicity, 1247
reversed 8-azaguanine carcinostasis, 935
Guanylic acid, protective against 6-mercaptopurine, 1247
reversed 8-azaguanine carcinostasis, 935

H-*2* locus. *See* Histocompatibility-2 locus
Hair, disulfides and sulfhydryls in, 769
growth stages, sulfhydryls in skin during, 341

Harderian gland, grafts, effects of vascularization on, 819
Heart, chick-embryo. *See under* Embryos
Hematopoiesis, extramedullary, in mice, 1343
granulocytic leukemia and, 1343
Hematopoietic tissue, in mice:
anatomy and pathology, 1281
morphology, histology, 1296
response to stress, 1307
review of literature, 1283
See also Bone marrow; Reticular tissue
Hepatomas, induced by feeding bentonite, 65
See also Liver, tumors of
Heredity. *See* Genotype
Histochemistry, of mammary tumorigenesis, 1123
Shope fibroma cell inclusions, 355
sulfhydryls and disulfides, 769
sulfhydryls in skin, 341
Histocompatibility, of tumor grafts:
changes induced in, 723
See also Adaptation
chromosome ploidy and, 723
Histocompatibility-2 locus, alleles at:
analysis and strain distribution, 457
growth of tumor grafts and, 485, 691
in mutant substrains, 481
HN_2. *See* Mustards, nitrogen
Hodgkin's disease, clinical response to α-peltatin, 1257
"Hodgkin's-like lesion," in mice, 1358
Homografts. *See* Grafts
Hormones. *See* ACTH; Adrenal cortex; Cortisone; Desoxycorticosterone; Gonadotrophin
HS. *See* Mustards, sulfur
Hyaluronidase, effect on filtrability of embryo extract, 189
Hydatid moles. *See* Moles
7-Hydroxy-2-acetylaminofluorene, method for measurement, 279
Hyperplasia, mammary, alkaline phosphatase in, 1123
of reticular tissue in mice, 1313
of mast cells, 1368
Hypophysectomy, effect on radioiodide in thyroid tumors, 593
Hypotension, podophyllin-induced, relation to tumor damage, 879

Immunity (acquired), to grafts, 705
vascularization and, 819
to tumor grafts, 537
mobile resistance factor, 537, 1277
Inclusions, in Shope fibroma cells, 355
Infectivity, of avian leukosis virus:
relation to particle count, enzyme activity, 1039
titration, 1055
of Mengo virus in ascites tumors, 627
Injury, reactions of mouse reticular tissues to, 1314
Intestine. *See* Gastrointestinal tract
Iodine. *See* Radioiodide
Irradiation. *See* X radiation
Isogenic resistant lines (of mice), histocompatibility loci and, 691
Isorhamnetin, 986

Keratinization, and sulfhydryls in skin, 341
disulfides and, 769
Keratosis, of buccal mucosa, relation to leukoedema, leukoplakia, 423
Kidney, disulfides and sulfhydryls in, 769
of rabbit, antigens compared with tumor antigens, 395

Lanthanum, effects on ascitic mice, 45
Leukemias:
human, chemotherapy agents on *in vitro* dehydrogenase, 1147
myelokentric and lymphokentric acids from blood, 439
response to α-peltatin, 1257
in mice, 1318
adaptation to strains, 1187
granulocytic, 1346
extramedullary hematopoiesis and, 1343
lymphocytic, 1322
See also Lymphocytic neoplasms
mast-cell, 1368
monocytic, 1351
plasmacytic, 1366
stem-cell, 1321
incidence in strain ST, 375
thymic, 1333
#1210, enzyme affected by chemical agents, 177
Leukocytes in mice, 1291
hormone effects on, 1307
hyperplasia of, 1313
neoplasms of, 1321
Leukoedema, of buccal mucosa in man, 423
Leukoplakia, relation to leukoedema, 423
Leukosis, avian erythromyeloblastic. *See under* Virus
Lipid. *See* Phospholipid

Lipopolysaccharide, and tumor-hemorrhagic activity of *E. coli*, 1195
Liver, changes in mice fed bentonite, 57, 65
glycogen content of cell strain from, 655
tumorigenesis in, effect of adrenal gland on, 805
tumors of, induced with fetal-tissue grafts, 1137
in rats fed *p*-dimethylaminobenzene-1-azo-2-naphthalene, 571
reticuloendotheliomas, 1351
Lung, transplanted, urethan carcinogenesis in, 411
See also Pulmonary tumors
Lymphatic tissue. *See* Reticular tissue
Lymph nodes, in cancer patients, response to α-peltatin, 1257
in mice, 1281, 1303
degenerative and inflammatory changes, 1311
effect of bone marrow on X-ray injury, 303
hyperplasia of cell types in, 1313
See also Reticular tissue
Lymphocytes in mice, 1294
neoplastic (studies on), 1334
protected from X ray by spleen shielding, 291
Lymphocytic neoplasms in mice, 1322
diagnosis, pathology, etc., 1337 ff
incidence, 1372 ff
review of literature, 1323
See also Lymphomas
Lymphokentric acid, from blood of leukemia patients, 439
Lymphomas, ascites, endomitosis in, 1
human, dehydrogenase affected by chemotherapy agents, 1147
response to α-peltatin, 1257
mediastinal, induced by oral methylcholanthrene, 329
regression with various X-ray treatments, 527
X-ray-induced, inhibited by bone-marrow injections, 303
prevented by spleen shielding, 291
#1 and #2, cytochrome oxidase affected by chemical agents, 177
See also Lymphocytic neoplasms
Lyophilized tissue, injected, effects on tumor grafts, 745

Mammary glands, response to gonadotrophin, B_6 deficiency, restricted diet, 493
Mammary tumor agent, and mammary tumor distribution, 895
in father, tumor incidence of hybrids, 317
Mammary tumors:
alkaline phosphatase activity during induction, 1123
delayed by weight reduction, 1253
distribution among glands, factors influencing, 895
in agent-free mice given methylcholanthrene orally, 329
incidence in ST mice, 375
in hybrids from agent-free mothers, 317
See also under Adenocarcinomas; Carcinomas
Mast cells, tissue (in mice), 1368
neoplasms of, 1368
Mediastinum, tumors of. *See under* Lymphomas
Megakaryocytes, in mice, 1295
Melanomas:
human, chromosomes *in vitro*, 905
phenoloxidase in, 1081
oxygen affinity compared with potato enzyme, 1093
S-91, cytochrome oxidase affected by chemical agents, 177
Melanoplus differentialis. *See* Embryos, grasshopper
Mengo encephalitis. *See under* Virus
6-Mercaptopurine, protection against toxicity of, 1247
"Mesenteric disease" in mice, 1312
incidence, 1375
Metabolism, of acetylaminofluorene in rats, 93, 279
rate of, effect on radiosensitivity in grasshopper embryos, 941
Methyl bis(2-chloroethyl) amine hydrochloride. *See* Mustards
Methylcholanthrene, tumor induction by: effect of age, 547
of croton oil, 83
in fetal-tissue grafts, 1137
in glandular stomach, 105
intravenous, in ST mice, 375
oral, in agent-free mice, 329
Milk agent. *See* Mammary tumor agent
Mitosis, in ascites tumor, effect of Mengo virus on, 627
See also Endomitosis
Moles, hydatid, gonadotrophin secretion in tissue culture, 1173

Monkeys, rhesus, gastric biopsy of, 141
Monocytes in mice, 1294
See also Reticulum cells
Monocytic leukemia in mice, 1351
Morphogenesis, effect of X rays on, 941
Mucosa, buccal, leukoedema of, 423
Muscle, fetal, tumor induction with grafts of, 1137
Mustards, nitrogen and sulfur, tumors induced by subcutaneous injection, 131
Mutation, at *H-2* locus, substrains from, 481
Myeloid leukemia. *See* Granulocytic leukemia
Myelokentric acid, from blood of leukemia patients, 439
Myeloma, clinical response to α-peltatin, 1257

N^{15}, distribution and excretion in rats given AAF-N^{15}, 93
Nerves, clinical transplantation, 669
disulfides and sulfhydryls in, 769
Neutrophils, in mice, 1292
Nitrite test, for measuring 7-hydroxy-2-acetylaminofluorene, 279
Nitrogen, in normal and cancer sera, 1233
in Shear's polysaccharide, 1099
recovery from rats given AAF-N^{15}, 93
diazotization method, 93
nitrite test, 279
Nitrogen mustard. *See* Mustards
Nucleic acid constituents, effect on 8-azaguanine carcinostasis in rabbits, 935
Nutrition, deficiency caused by bentonite feeding, 57
See also Culture media; Diet

Oncolysis. *See* Tumor-damaging agents
Ovaries, response to gonadotrophin, B_6 deficiency, restricted diet, 493
tumors of, induced by transplantation to old castrated mice, 77
Oxidizing enzymes, in human melanomas, 1081
Oxygen, affinity of human and potato phenoloxidase for, 1093

Papillomas, effect of croton oil on, 83
induced by mustards, 131
Parabiosis, protective against X ray, 149, 159
Peltatins:
α-, effects on cancer patients, 1257
α- and β-, chemistry of, 986
effect on tumor enzyme, 177
Peptides, in embryo extract, utilization by heart cultures, 519
Peyer's patches, in mice, 1281, 1307
Phenanthrenes, effect on Sarcoma 37, 365
Phenazines, effect on tumor cytochrome oxidase, 177
Phenoloxidase activity, of human melanomas, 1081
oxygen affinity compared with potato enzyme, 1093
Phospholipid, in Shear's polysaccharide, 1099
Picropodophyllin, chemistry of, 986
Picropodophyllin glucoside, 986
Plant materials, tumor-damaging, 607
Plasma. *See* Blood plasma
Plasma cells in mice, 1294
hyperplasia, 1313
neoplasms, 1366
Platelets, in mice, 1295
Podophyllic acid, chemistry of, 986
Podophyllin, biological effects (review), 967
chemistry of (review), 986
effects on blood pressure, 879
See also Peltatins
Podophylloresin, 986
Podophyllotoxin, chemistry of, 986
effects on blood pressure, 879
on tumor enzymes, 177, 585
Polysaccharide (Shear's), chemistry of, 1109
fractionation, 1099
physicochemical characteristics, 1119
See also Lipopolysaccharide
"Potter lesion," in mice, 1364
Precancerous lesions, induced in glandular stomach, 105
Proceedings: Tissue Transplantation Conference, 665
Promoting action. *See under* Tumorigenesis
Propylthiouracil, and radioiodide in thyroid tumors, 593
Proteins, in culture media, 513
utilization by heart cultures, 519
in Shear's polysaccharide, 1099, 1109
in Shope fibroma cell inclusions, 355
sulfhydryl and disulfide groups, histochemistry, 769
sulfhydryl groups in skin during hair growth, 341
See also Albumin; Globulin
Pulmonary tumors (in mice):
dibenzanthracene-induced, strain susceptibility, 127

Pulmonary tumors (in mice)—*Continued*
incidence increased by subcutaneous HN_2, 131
incidence in strain ST, 375
induced by oral methylcholanthrene, 329
urethan-induced in lung grafts, 411
Purines, effect on 8-azaguanine carcinostasis in rabbits, 935
ribotides, protective against 6-mercaptopurine, 1247
Pyridoxine deficiency, effects on gonadotrophin-injected and/or diet-restricted mice, 493

Quercetin, from podophyllin, 986
Quinoxyline derivative, effect on tumor cytochrome oxidase, 177

Radiation. *See* X radiation
Radioactive amino acids, uptake by culture cells, 519
Radioiodide, concentration in thyroid tumors, 593
Radiolanthanum, effects on ascitic mice, 45
Radiosensitivity:
of human cancer tissue, dehydrogenase activity and, 1147
relation to metabolic rate, 941
Research, cancer, emphasis in, 1205
Resistance, acquired. *See* Immunity
to arsenicals, of tumor-bearing and normal mice, 1223
Respiration, tumor, effects of tumor-damaging agents on, 585
Reticular tissue (in mice), anatomy and pathology, 1281
degenerative and inflammatory changes, 1311
hyperplasia of cell types, 1313
neoplastic conditions, 1318
incidence, 1372 ff.
relation to human neoplasms, 1373
organs of, 1296
response to hormones, 1307
to injury, 1314
survey of literature, 1283
Reticulum cells in mice, hyperplasia, 1313
neoplasms, 1350
incidence, 1372 ff.
Ribotides. *See under* Purines
Rotation velocity, effect on fluid-suspension cultures, 1159
effect on surface substrate cultures, 853
Russell's bodies, in mouse cells, 1313

Sarcoma 37:
damaged by chemical agents, 365, 645
by plant materials, 607
effect on resistance to arsenicals, 1223
enzyme changes induced by chemical agents, 177, 585
growth in various mouse stocks, 449
H-2 allele and, 485
Sarcoma 180, *H-2* allele and strain specificity, 485
Sarcomas, ascites, endomitosis in, 1
growth curves of, 229
damaged by podophyllin, hypotension and, 879
human, chromosomes *in vitro*, 905
dehydrogenase affected by chemotherapy agents, 1147
induced in glandular stomach, 105
reticulum-cell, 1351
subcutaneous, effects of age on induction, histology, 547
induced by mustards, 131
See also Fibrosarcomas
Sedimentation, of Shear's polysaccharide, 1119
of virus particles and enzyme, 1027
and infectivity, 1039
Serratia marcescens, tumor-necrotizing agent from, chemistry of, 1109
fractionation, 1099
physicochemical characteristics, 1119
Serum. *See* Blood serum
Shear's polysaccharide. *See* Polysaccharide
Shope fibroma. *See* Fibroma
Sikkimotoxin, 986
Skin, effects of podophyllin on diseases of, 967
grafts, clinical, 669, 683, 689, 704, 718, 741
fetal, tumor induction with, 1137
immunity to, 705
sulfhydryls in, during hair growth, 341
tumors of. *See* Papillomas
Spectrophotometry, for measuring 7-hydroxy-2-acetylaminofluorene, 279
Spleen, anatomy and pathology in mice, 1281, 1296
effect of bone marrow on X-ray damage to, 303
in lymphoma patients, response to α-peltatin, 1257
shielding of, effect on thymic recovery from X rays, 303
prevents X-ray-induced lymphomas, 291

Spleen—*Continued*
tumors in, in ovarian implants, 77
induced with fetal-tissue grafts, 1137
See also Reticular tissue
Stem-cell leukemia, in mice, 1321
Stilbestrol, mammary tumors induced by, 1123
Stomach:
fetal, tumors induced with grafts of, 1137
glandular, methylcholanthrene-induced tumors in, 105
instrument for biopsy of, 141
lesions in ST mice, 375
See also Gastrointestinal tract
Stress, effects on reticular tissues in mice, 1307
Succinoxidase, effects of tumor-damaging agents on, 585
Sugars, in Shear's polysaccharide, 1109
Sulfhydryls, histochemistry, 769
in skin, hair growth and, 341
in normal and cancer sera, 1233
Sulfite, action on disulfide in normal and cancer sera, 1233
Sulfur mustard. *See* Mustards

Teeth, lamprey, disulfides and sulfhydryls in, 769
"T" flasks, for tissue cultures, 841
Thiouracil, thyroid gland tumors and, 593
Thymus in mice, 1281
effect of bone marrow on recovery from X rays, 303
hormone effects on, 1307
leukemia originating in, 1333
size and morphology, 1301
See also Reticular tissue
Thyroid gland:
grafts of, vascularization and, 819
tumors of, radioiodide-concentrating ability, 593
Tissue cultures, fluid-suspension, 1159
glycogen content in 2 cell strains, 655
of human skin, 741
of human tumors, chromosomes in, 905
of hydatid moles, gonadotrophin secretion in, 1173
of mouse leukemias (review), 1335
surface-substrate, effect of rotation velocity, 853
"T" flasks for, 841
utilization of amino acids, 519
See also Cell proliferation; Culture media
Tissues, disulfides and sulfhydryls in, 769
effect of X rays on differentiation, 941
fetal, tumors induced with grafts of, 1137
of tumor-bearing mice, distribution of injected arsenic in, 1223
rat, AAF distribution in, 93, 279
See also names of tissues
Tissue Transplantation Conference, Proceedings of, 665
Toxicity, of 6-mercaptopurine, protection against, 1247
of α-peltatin, reactions of cancer patients to, 1257
Trachea, clinical transplantation, 669
disulfides and sulfhydryls in, 769
Transmission, of avian leukosis:
strain differences, 1067
titration of virus, 1055
Transplantation:
leukemia, strain adaptation, 1187
tissue, clinical aspects, 669
conditioning the host, 745
conference on, Proceedings, 665
tumor, acquired immunity to, 537, 705
mobile resistance factor, 537, 1277
chromosome ploidy and, 723
conditioning the graft, 723
histocompatibility genes and, 457, 481, 485, 691
See also Grafts
Transplantation Bulletin, announcement of, 227
Triethylene melamine, effect on dehydrogenase of human cancer tissue, 1147
Tropolones, effect on Sarcoma 37, 365
Tumor-damaging agents:
bacterial. *See Escherichia coli*; *Serratia marcescens*
chemical, on Sarcoma 37, 365, 645
effects on enzyme activity, 177, 585
on *in vitro* dehydrogenase of human tumors, 1147
from plant materials, 607
See also α-Peltatin; Podophyllin; Virus, Mengo encephalitis
Tumorigenesis:
alkaline phosphatase during, 1123
delayed by weight reduction, 1253
effect of age on, 547
in glandular stomach of mice, 105
lymphocytic, in mice, 1329
ovarian, in old mice, 77
promoting action of croton oil, 83
See also Carcinogens

Tumors:
adaptation induced in. *See* Adaptation
ascites. *See* Ascites tumors
cells of. *See* Cells
damage. *See* Tumor-damaging agents
effects of podophyllin on, 879, 967
human, chromosomes *in vitro*, 905
effect of chemotherapy agents on dehydrogenase, 1147
nitrogen, sulfhydryl, disulfide in sera, 1233
response to α-peltatin, 1257
immunity to. *See under* Immunity
incidence in ST mice, 375
in rats protected against lethal X-ray doses, 149, 159
lung. *See* Pulmonary tumors
mammary. *See* Mammary tumors
subcutaneous. *See under* Sarcomas
transformation of solid to ascites, 723
transplantation. *See under* Transplantation
See also Adenocarcinomas; Ascites tumors; Carcinomas; Fibromas; Hepatomas; Leukemias; Lymphomas; Melanomas; Papillomas; Sarcomas
See also under Epithelium; Gastrointestinal tract; Liver; Ovaries; Reticular tissue; Spleen; Stomach; Thyroid gland
Tyrosinase, in human melanomas, 1081

Ultracentrifugation, of embryo extract for culture media, 189
See also Sedimentation
Urethan, carcinogenic effect on transplanted lung tissue, 411
effect on dehydrogenase of human cancer tissue, 1147
Uric acid, excretion in patients given α-peltatin, 1257
Urine (rat), recovery of acetylaminofluorene from, 93
measurement, 279
Uterus, response to gonadotrophin, B_6 deficiency, restricted diet, 493

Vascularization, of grafts, 687, 719
host resistance and, 819
Virus:
avian leukosis:
particles, and enzyme activity, 1011
electrophoresis, sedimentation, 1027
infectivity and, 1039
strain variation in response, 1067
titration, 1055
Mengo encephalitis, effects on ascites tumors, 627
Viscosity, effect on fluid-suspension cultures, 1159
Vitamins, B_6. *See* Pyridoxine
supplements in culture media, 513

Weight, effect of pyridoxine deficiency on, 493
reduction, and rate of tumorigenesis, 1253

X radiation:
effects on grasshopper development, 941
on reticular tissue in mice, 1316
lymphomas induced in mice by, 1329
inhibited by bone marrow, 303
prevented by spleen shielding, 291
protection against, by *p*-aminopropriophenone, 159
by bone injections, 955
by glutathione, 159
by parabiosis, 149, 159
systemic and/or local, dose-responses of tumor regression, 527
thymic recovery aided by bone-marrow injections, 303
tumor immunity and, 720, 745
tumors induced in protected rats by, 149, 159
See also Radiosensitivity
XYZ factor, 745

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CONTENTS

JOURNAL OF THE NATIONAL CANCER INSTITUTE • VOL. • 14 • NO. • 6 • JUNE • 1954

Page

Table of contents, Volume 14 ... V

Emphasis in cancer research. *Paul E. Steiner* ... 1205

Effect of growth of mouse Sarcoma 37 on resistance of mice to semilethal doses of certain trivalent arsenic compounds. *Lyle V. Beck* and *Ruth Joyce Gillespie* ... 1223

Nitrogen, sulfhydryl, and disulfide contents in normal and cancer sera and in their albumin and globulin fractions. Action of sulfite on disulfide groups in normal and cancer sera. *I. M. Kolthoff, W. Stricks, Loes Morren,* and *A. Heyndrickx* ... 1233

Demonstration of protection against the lethal toxicity of 6-mercaptopurine in mice. *Abraham Goldin* ... 1247

The effect of weight reduction on the occurrence of spontaneous mammary tumors in mice. *Samuel H. Wexler* ... 1253

Response of patients with advanced neoplasms to the intravenous administration of ***alpha***-peltatin. *Ezra M. Greenspan, Jacob Colsky, Emanuel B. Schoenbach,* and *Murray J. Shear* ... 1257

Mobility and site of production of homograft resistance factor. *Richmond T. Prehn* and *Joan M. Main* ... 1277

Normal and pathologic anatomy of the reticular tissue in laboratory mice, with a classification and discussion of neoplasms. *Thelma B. Dunn* ... 1281

Index of authors, Volume 14 ... 1435

Index of subjects, Volume 14 ... 1437